GENERAL SURVEY, COLLECTION, EXTRACTION, PHYTOCHEMISTRY AND ANTIMICROBIAL ACTIVITY OF SOME MEDICINAL PLANTS

2.1. General survey on medicinal plants

India has prosperous traditions of medicinal herbs and shrubs, which consist of about more than 2000 species and has an enormous geographical region with high prospective abilities for Ayurveda, Siddha, and Unani and other traditional medicines, but only very few have been studied chemically and pharmacologically for their potential medicinal value [1,2]. According to the World Health Organization (WHO), most populations still rely on traditional medicines for their psychological and physical health requirements [3]. Rural areas of many developing countries still depend on traditional medicine for their primary health care needs and have found a place in day-to-day life. These medicines are comparatively safer and cheaper than synthetic or modern medicine [4-6]. People living in rural areas from their own experience know that these traditional remedies are a valuable source of natural products to balance human health, but they may not understand the science behind these medicines, but knew that some medicinal plants are highly effective only when used at therapeutic doses [7, 8]. Herbal medicines are in great demand in both developed and developing countries as a source of primary health care owing to their attributes having wide biological and medicinal activities, high safety margins and lesser costs. Herbal molecules are safe and would overcome the resistance produced by the pathogens as they exist in a combined form or in a pooled form of more than one molecule in the protoplasm of the plant cell [9, 10]. The traditional use of herbal medicine is recognized as a way to learn about potential future medicines. Researchers have identified a number of
compounds used in mainstream medicines which were derived from "ethnomedical" plant sources [11].

The medical management of Alzheimer’s disease (AD) includes medications such as acetylcholinesterase (AChE) inhibitors and N-methyl-di-aspartate (NMDA) receptor antagonists. Based on the literature review, the conventional drugs is statistically significant but clinically significance is limited to the slowing, rather than a reversal of the disease.

Phytochemicals are the chemical molecules commonly found in plants, not usually processed for pharmacological purposes. Phytochemicals influence the function of various receptors for both excitatory and inhibitory neurotransmitters in the brain and thus can maintain or alter the chemical balance of the brain. This knowledge was used in the traditional practice of medicine after several plants were identified to have medicinal properties to treat cognitive disorders. A growing number of herbal remedies are available in the market claim to be effective as mood enhancers, memory boosters or agents to slow down or prevent Alzheimer’s disease and related dementias. There are few objective measures to evaluate the safety and effectiveness of these products. The effectiveness of these drugs is based largely on tradition and a rather small body of scientific research. Law for the marketing of dietary supplements does not require the rigorous scientific research and clinical trials by the U.S. Food and Drug Administration (FDA) for the authorization. Indeed, numerous scientific studies have explained the use of various Ayurvedic medicinal plants named ‘nervines’ and their constituents to reinforce the functional activity of the refurbishment of memory and a nervous system [12,13]. Up to now, efforts to discover a cure for AD has been unsatisfactory and drugs presently available to treat Alzheimer’s disease address merely its symptoms and with partial effectiveness.

The present assessment places collectively research on various Ayurvedic medicinal plants that have shown promise in reversing the Alzheimer’s disease pathology. The literature survey summarizes information regarding the phytochemical and biological and clinical applications of these various plants in order to provide adequate baseline information that could be used in drug discovery operations and development, thereby providing new functional leads for AD. In our research, we aim to put focus particularly on the most prescribed herbal medicines and compound from plants used in the treatment of AD. The various medicinal plants that are suggested for AD and their actions on the brain are as follows.

**Withania somnifera** (Ashwagandha)

*Withania somnifera* also known as Ashwagandha is a member of a *solanaceae* family, and the root part was widely used. It is used extensively in Ayurveda as a nervine tonic and adaptogen and assists the body to adapt to stress [13]. It is an agent that promotes cognition and memory. A total alkaloid extract of ashwagandha root showed a calming effect on the central nervous system (CNS) in several mammalian spices, suggesting the use of this herb to produce relaxation. Aqueous extracts of this herb have been found to increase in the cholinergic activity, including increases in the acetylcholine content and choline acetyltransferase activity in the rats this might partly explain the cognition-enhancing and memory-improving effects[14].

**Bacopa monniera** (Brahmi)

*Bacopa monniera* (also known as Brahmi) is a bitter-tasting creeper plant found in damp and marshy regions and is commonly used in Ayurvedic medicine as a nerve tonic, diuretic, and cardiotonic and as a remedial agent against epilepsy, asthma,
rheumatism, and insomnia [15,16]. By tradition, *B. monniera* was used to improve memory and cognitive function [17]. Brahmi also inhibited cholinergic degeneration and shows cognition-enhancing results in a rat model of Alzheimer’s disease [18].

**Caprylic acid from coconut**

Caprylic acid is a medium-chain triglyceride and it is the key constituent of Axona-a medicinal food advertised for Alzheimer’s disease produced from the coconut or palm kernel by processing. Caprylic acid is changed to ketone bodies when metabolized and ketone bodies act as alternate energy origin in the brain [19].

**Centella asiatica**

*Centella asiatica* is a psychoactive therapeutic plant used for centuries in the Ayurvedic method of medicine as a constituent of medhya rasayna. It has been shown to reduce the oxidative stress in the brain [20]. Major bioactive compounds of this plant enclose highly variable related sapogenins and triterpenoid saponins [21]. Asiaticoside derivatives, including Asiatic acid and asiaticoside, were an exhibit to reduce hydrogen peroxide-induced cell death, reduce free radical concentrations, and inhibit beta-amyloid cell death *in vitro*, signifying a possible role for *C. asiatica* in the treatment and prevention of Alzheimer’s disease and beta-amyloid toxicity.

**Tinospora cordifolia**

*Tinospora Cordifolia* is a member of a *Menispermaceae* family, possesses a memory enhancing property for learning and memory in normal and memory-deficits animals. The mechanism for cognitive improvement is by immunostimulation and synthesis of acetylcholine, this supplementation of choline enhances the cognitive function [22].

**Magnolia officinalis**

*Magnolia officinalis* is a member of a *Magnoliaceae* family, commonly called houpu magnolia. The bark of this tree is used as a traditional memory enhancing agent in
Chinese medicine for the treatment of anxiety, neurosis, stroke and dementia. *M. officinalis* inhibits the memory impairment induced by scopolamine through the inhibition of AChE [23].

**Convolvulus pluricaulis**

*Convolvulus pluricaulis* (shankhpushpi) is a common plant in India, where the whole plant is used in various formulas as a nervine tonic for improvement of memory and cognitive function [24]. It is believed that shankhpushpi calms the nerves by regulating the body's production of the stress hormones, adrenaline, and cortisol. The ethanolic extract of *C. pluricaulis* and its ethyl acetate and aqueous fractions significantly improved learning and memory in rats [25].

**Celastrus paniculatus**

*Celastrus paniculatus* is a member of a *Celastraceae* family, commonly known as jyotishmati, is a treasured medicinal herb that is revered for its effects on the brain and has been used for centuries in Ayurveda for sharpening the memory and improving concentration and cognitive function. Aqueous extracts of *C. paniculatus* seed have dose-dependent cholinergic activity, thereby improving memory performance [26].

2.1.2. Clinically used herbal originated drugs in Alzheimer’s disease treatment.

2.1.2.1. *Ginkgo biloba L.*

*Ginkgo biloba L.*, also known as a maidenhair tree, *G. biloba* (*Gingkoceae*), one of the oldest plants living on earth with geological records indicating this plant has been growing on earth for 150-200 million years. It is also one of the best-known medicinal plants with traditional use being recorded as early as 2800 BC in the traditional Chinese medicine (TCM). *G. biloba* was first introduced to Europe in 1690 by an ethnobotanist Engelbert Kaempfer [27]. The drugs that have been prescribed until
now most often in Germany against dementia are the extracts of *G. biloba* and it was the second best selling nutritional supplement in the USA [28]. It was reported that about 7 billion dollars are spent yearly on herbal medicines and *G. biloba* ranks first among herbal medications [29]. In France, China and USA, 50 million Ginkgo trees are cultivated, which generate more than 8000 tons of dried leaves yearly to meet the saleable demand for *G. biloba* preparations [30]. Considering the involvement of oxidative stress and spreading free radical reactions in its pathology, Alzheimer’s disease is one of the best characterized neurological diseases [31-33]. Because in dementia based on impaired neurotransmission and neuronal loss a reduction in oxygen and glucose accompanied with the progress of free radicals and lipid peroxidation has been observed [34]. Furthermore, a relationship between the formation of hydrogen peroxide, accumulation of reactive oxygen species (ROS) and oxidative stress (OS) and AD has been again mentioned [35]. However, the plant generally contains flavonoid derivatives such as mono, di-, and tri-glycosides of quercetin and kaempferol; biflavonoids including amentoflavon, ginkgetin and isoginkgetin; trilactonic diterpenes such as ginkkolides A, B, C, J (Fig.2.1.1.) [36, 37]. Numerous investigations have been conducted concerning the potential of *G. biloba* in cognitive disorders. More recently, an in vitro study point toward that the extract had also an anti-amyloid aggregation effect suggesting another mechanism whereby *G. biloba* may be effective in preventing or delaying the development of Alzheimer’s disease [38]. A quite number of clinical trial studies have been carried out on Alzheimer’s disease patients with *G. biloba* presenting the optimistic effect of this herbal medicine in cognitive deficits [39, 40]. These studies underlined that the efficiency of *G. biloba* in Alzheimer-type of dementia is likely to result from a synergistic interaction between flavonoids and terpene lactones. Recent evidence also
suggests that it may have direct effects on the cholinergic system which might help to clarify both its acute and chronic cognitive-enhancing effects.

![Chemical structures of gingkolides A, B, C and J](image1.png)

**Fig.2.1.1. Chemical structures of gingkolides A, B, C and J**

### 2.1.2.2. Galantamine

Galantamine is an isoquinoline alkaloid isolated firstly from *Galanthus woronowii* belongs to *Amaryllidaceae* family commonly known as “snowdrop” (Fig.2.1.2.) [41].

It was later on also isolated from some other members of the *Amaryllidaceae* family including *Narcissus* and *Leucojum aestivum*, in which we showed the presence of galanthamine and other similar-structured isoquinoline alkaloids and determined their Acetylcholinesterase (AChE) inhibitory effects [42]. Positive AChE inhibitory effects of several *Amaryllidaceae* alkaloids having galanthamine and lycorine skeletons were also reported [43].

![Chemical structure of galanthamine.](image2.png)

**Fig.2.1.2. Chemical structure of galanthamine.**
Galanthamine has been found to be the long-acting and specific inhibitor of AChE enzyme and to potentiate cholinergic nicotinic neurotransmission by allosterically modulating nicotinic acetylcholine receptors which may be of additional value in the treatment of AD [42, 44, and 45]. Acetylcholinesterase (AChE) inhibitors such as physostigmine, galanthamine, eptastigmine, and metrifonate the relation between AChE inhibition and cognitive effect seem to be different than other AChE inhibitors including tacrine and donepezil in terms of efficacy and adverse effects. Although the most common side effect observed after galanthamine administration is nausea, it is possible to eliminate nausea by increasing the galanthamine dose slowly [46]. Galanthamine was revealed to be safe from the viewpoint of hepatotoxicity [47]. Another advantage of galanthamine is its reversible and competitive inhibition of AChE. All these studies have revealed that galanthamine, a potent inhibitor of AChE, is an efficient commercial drug used in the treatment Alzheimer’s disease. This natural phytocompound can be taken as a replica to develop novel AChE inhibitors for future studies.

2.1.3. Future -Promising herbal originated molecules in AD treatment.

2.1.3.1. Huperzine A

*Huperzia serrata* is one of the genera in a *Huperziaceae* family (syn. *Lycopodiaceae* family). This genus, used for its memory-enhancing effect since ages in TCM, is well-known to contain a large group of alkaloids called “Lycopodium alkaloids” [48]. The plant, known as “Qian Ceng Ta” in Chinese, is mostly grown in subtropical regions of the world as well as in China naturally along the Yangtze River as well as the southern parts of the country. Huperzine A is a quinolizidine alkaloid, firstly isolated from *H. serrata* at 1986 by the Chinese researchers at Institute of Materia Medica in Shanghai (Fig. 2.1.3.) [49,50]. The series of alkaloids named as Huperzine A to P
were isolated by different research groups, however, only Huperzine A sparkled for its influential AChE inhibitory among them [50].

![Chemical structure of Huperzine A](image)

**Fig. 2.1.3. Chemical structure of Huperzine A**

Apart from its in vitro activity, this compound was shown to display in vivo potent, selective, and reversible inhibitory effect on AChE using various experimental models in rats [51-53]. The activity of Huperzine A has been found to be as high as physostigmine, galanthamine, donepezil, and tacrine, the commercial drugs already used against the AD, or even greater [54, 55]. Huperzine A was compared to donepezil and tacrine on the radial maze performance in ethylcholine mustard aziridinium ion-treated rats and the results showed that Huperzine A had a higher bioavailability and more selective inhibition on AChE activity in rat cortex and hippocampus. In 1996, a tablet form of Huperzine A named as “shuangyiping” was developed for Alzheimer’s treatment in China [56,57]. The treatment of Alzheimer’s disease patients with Huperzine A compound caused delaying hydrolysis of acetylcholine and enhancement the level of the neurotransmitter called acetylcholine in the synaptic cleft. The above-mentioned literature data cover that Huperzine A is the most promising drug of herbal origin which will become accessible in the market in a near future to treat AD.

### 2.1.3.2. Terpenes and Diterpenes from Salvia species

Salvia species a member of a *Lamiaceae* family, referred to “sage” in English, is well-known to enhance memory in European folk medicine [58]. Consequently, a number of studies have been so far performed on various species of this genus
considering their anti-AChE potentials. Investigated AChE inhibitory effect of the essential oil obtained from *Salvia lavandulaefolia* using human erythrocyte AChE by spectrophotometric Ellman method and analyzed the chemical composition of the essential oil by gas chromatography-mass spectrometry (GC-MS). The oil was found to contain a number of monoterpenes such as (+)-α-pinene, α- and β-terpineol, citronellal, γ-terpinene, R-(+)-limonene, 1,8-cineole, 1R-(+)-camphor, linalool, 1S-(-)-β-pinene, and geraniol. *S. officinalis* was also analyzed in that study applying the same method. As a result, pure forms of (+)-α-pinene and 1,8- cineole were independently tested (Fig. 2.1.4.) and concluded that synergistic interactions of these two simple molecules have caused the high Anti-AChE activity of the two *salvia* essential oils mentioned [59].

\[\text{α-Pinene} \quad \text{1, 8-cineole}\]

**Fig. 2.1.4. Chemical structure of Terpenes from Salvia species**

Alternatively, the roots of *Salvia miltiorrhiza*, a well-known Chinese plant, has been also found to afford some diterpene compounds with AChE inhibitory activities including tanshinone I and IIA, cryptotanshinone, and 15,16- dihydrotanshinone (Fig. 2.1.5) [60]. In a recent study tanshinone I, tanshinone IIA, cryptotanshinone, and 15, 16-dihydragranthinone I were tested in mice on learning and memory impairments induced by scopolamine using passive avoidance tasks, where tacrine was the reference drug [61]. The tested diterpenoids were effective in reversing scopolamine-induced cognitive impairments. Tanshinone I and Tanshinone IIA were also reversed
diazepam-induced cognitive dysfunctions.

![Chemical structure of diterpenes from *Salvia miltiorrhiza*.](image)

**Fig. 2.1.5.** Chemical structure of diterpenes from *Salvia miltiorrhiza*.

### 2.1.3.3. Alkaloids from various plant species.

Alkaloids, the nitrogen-containing compounds, have been normally observed to have a strong inhibition of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). Among the different classes of alkaloids, isoquinoline alkaloids have been reported to possess anti-acetylcholinesterase activity. The most well-known anti-AChE example of this class is galanthamine as mentioned in above parts. A preliminary screening study screened a number of Turkish plants for their anticholinesterase (AChE) activity [62]. Among them, ten *Fumaria* species of *Fumarioideae* subfamily of *Papaveraceae*, which are *F. asepala* Bois, *F. capreolata* L, *F. ciliicica* Hausskn, *F. densiflora* DC, *F. judaica*, *F. kralikii* Jordan, *F. macrocarpa* Parlatore, *F. parviflora* Lam, *F. petteri* subsp. *thurietii* (Boiss.) Pugsley and *F. vaillantii* Lois, by Ellman method. Besides, *F. vaillantii* Lois extract having 94.23% inhibition was applied to bioactivity directed fractionation in order to isolate the constituents responsible for the activity [63]. Through the active fractions of the extract 10 isoquinoline alkaloids...
were isolated by preparative thin layer chromatography (TLC). Among the alkaloids, ophiocarpine had the most potent inhibitory activity followed by β-allocryptopine, berberine, ophiocarpine-N-oxide, and protopine (Fig. 2.1.6.).

Another alkaloid, juliflorine isolated from Prosopis juliflora is a member of Fabaceae was found to inhibit both acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) in a concentration-dependent manner (Fig. 2.1.7)[64].

Even though AChE inhibitors seem to be the main choices for Alzheimer’s disease so far, new mechanisms are rising day by day since the pathogenesis of the disease has not been completely understood, up till now. There is still a need to develop more efficient drugs for Alzheimer’s disease. The medical and economic benefits of herbal medicinal products with health claims are growing worldwide. hope that all these research in finding new drug molecules for Alzheimer’s disease may still continue to
focus on plants as it has been and the compounds of herbal origin such as galanthamine and huperzine A as the model drugs may lead to discovery of novel drugs.
2.2. Collections of medicinal plants

Based on the literature survey of medicinal plants, three medicinal plants were selected and collected for the study of pharmacological application to alleviate Alzheimer’s disease. While selection process, medicinal plants on which there were no reports or very less reports of neuropharmacological and acetylcholinesterase (AChE) screening activity were selected. It was envisaged that a lot of scope still remained in the field of neurodegenerative disorder studies on the three plants that were selected. In view of conserving endangered species of flora, many government and non government organizations have set up medicinal plant gardens in this district Tumkur the spots where plants were collected below.

- “Siddha Sanjeevani Aushadhi Vana” at Namada Chilume, Devarayanadurga Tumkur district, maintained by the department of forest, Government of Karnataka.
- “Siddara betta medicinal plants garden” at Madhugiri, Tumkur district, maintained by the department of forest, Government of Karnataka.
- “Medicinal Plants Conservation Park” at Tiptur, Tumkur district, maintained by BAIF Institute for Rural Development (BIRD )

In a continuation of this research programme, first it was thought to collect medicinal plants useful in remedial of Alzheimer diseases and then subject them to various methods of extraction. Accordingly, first the study of antimicrobial properties and acetylcholinesterase activities of the plants was carried out and then the active plants were taken up for further separation, isolation, structural elucidation and their pharmacological activities. The plants were collected during their flowering seasons, from in and around tumkur district. All the selected plants were authenticated with assistance of department with assistance of the department of forest (Tumkur district).
The selected and collected plants were as follows.

2.2.1. *Alpinia Purpurata*.

2.2.2. *Mimusops elengi*.

2.2.3. *Rauvolfia tetraphylla*.

Considering the importance of plant driven compounds in drug discovery, the present research was undertaken to evaluate (extraction, isolation and structural elucidation of bioactive compounds) of above selected medicinal plants with various ethnobotanical uses, aiming to potential candidates for alleviate Alzheimer’s disease.
2.2.1. *Alpinia purpurata*

![Fig.2.2.1. *Alpinia purpurata*](image)

**Kingdom:** Plantae  
**Order:** Zingiberales  
**Family:** Zingiberaceae  
**Genus:** Alpinia  
**Species:** Purpurata  
**Common name:** Red ginger.  
**Habitat:** Rhizomes  

**Geographical distribution:** It is a native to the Pacific.

**General description:** It is a rhizomatous perennial, leafy herb, forming large clumps, growing to 3-4 meters tall. Leaves are lanceolate, glabrous. Inflorescence is a terminal spike, often pendulous, showy, up to 90 centimeters long. Flowers are small, white, arising from the red bracts of the spike, to 2.5 centimeters long. Fruit is a capsule. Pseudostems formed by leaf sheaths emerge from underground rhizomes.

**Parts used:** Leaves, flowers, fruit and rhizome.

**Medicinal uses:** Several species of the genus *Alpinia* have been reported to exhibit antimicrobial activity against certain microorganisms [65]. Its phytomedicinal
potential to treat tuberculosis is also described [66]. *A. purpurata* may serve as anticancer agents against ovarian cancer cell lines [67].

Phytochemical studies on *A. purpurata* revealed that, it possess flavonoids, rutin, kaempferol-3-rutinoside and kaempferol-3-glucuronide [68].

![Rutin](image1)

**Rutin**

![Kaempferol-3-rutinoside](image2)

**Kaempferol-3-rutinoside**

Fig. 2.2.2. Structures of various phytocompounds revealed from *A. Purpurata*. 
2.2.2. *Mimusops elengi*

![Mimusops elengi](image)

**Fig.2.2.3. Mimusops elengi**

- **Kingdom:** Plantae
- **Order:** Ericales
- **Family:** Sapotaceae
- **Genus:** Mimusops
- **Species:** Elengi

**Botanical Name:** *Mimusops elengi*

**Common name:** Bullet wood (English), Bakul (Sanskrit), Pagade Mara (Kannada).

**Habitat:** Tree

**Geographical distribution:** Throughout Asia, including India and Bangladesh.

**General description:** An evergreen tree reaching a height of about 16 m (52 ft). Leaves are glossy, dark green, oval-shaped, 5–14 cm (2.0–5.5 in) long, and 2.5–6 cm (0.98–2.36 in) wide. Flowers are cream, hairy and scented. Bark is thick and appears dark brownish black or grayish black in colour, with striations and a few cracks on the surface.

**Parts used:** Stem bark, leaves, flowers, fruit and seed

**Medicinal uses:** The different parts of the *M. elengi* plant (flowers, seeds, fruits and bark) have great medicinal value. The flowers, fruits and bark of this plant are used in
the treatment of diarrhea, dysentery [69]. Seed and fruit of *M. elengi* showed presence of quercitol, ursolic acid, dihydro quercetin, quercetin, β-d glycosides of βsitosterol and alphaspinasterol after saponification [70], Taraxerone, taraxerol, betulinic acid and spinasterol, sodium salt of betulinic acid, Fatty acid esters of alpha-spinasterol and ursolic acid was isolated from the bark [71]. Hentriacontane and carotene from the leaves, heartwood and roots were isolated. A steroidal saponin, 5-α-stigmast-9(11) en-3-obeta-D-glucopyranosyl (1-5)-o-beta-D-xylofuranoside was isolated from the roots of *M.elengi* [72,73]. The leaves contain sterols, reducing sugars and tannins [74]. Pulp of the fruit contains a large proportion of sugar and saponins [75]. Leaves are used as an antidote for snakebite [76].

**Fig.2.2.4.** Structures of various isolated phytocompounds from *M. elengi*. 
2.2.3. *Rauvolfia tetraphylla*

![Fig. 2.2.5. *Rauvolfia tetraphylla*](image)

**Kingdom:** Plantae  
**Order:** Gentianales  
**Family:** Apocyanaceae  
**Genus:** Rauwolfia  
**Species:** Tetraphylla  
**Common Name:** Be still tree  
**Habitat:** Bush or small tree.

**Geographical distribution:** The plant is native to Mexico, Central America and West Indies and is now naturalized throughout the tropics including Australia, Indo-china and India.

**General description:** *R.tetraphylla* is a small, tree or shrub that reaches 6 feet in height. Leaves are whorled, medium to dark green in colour; occur in groups of 4 equally-sized leaves.

**Parts used:** Leaves, fruits and roots.

**Medicinal uses:** *R. tetraphylla* is an economically important medicinal plant because of the presence of various indole alkaloids reported from roots [77] and different parts also. About 30 indole alkaloids are reported in *Rauwolfia* genus and reserpine holds
the first place among them. Other reported alkaloids are ajmalicine, aricine, reserpinine, reserpine, serpentine, isoreserpinine, tetraphylline, corynanthine, yohimbine, α- yohimbine, β- yohimbine and ψ- yohimbine [78]. (shown in fig.2.2.6.)

Reserpine is a potent alkaloid that depresses the lowers blood pressure and central nervous system. The leaf extract of *R. tetraphylla* is intended for the treatment of cholera, fever and eye disease. It is also used as antihypertensive, also in intestinal disorders, diarrhea and dysentery [79]. The methanolic leaves extract of *R. tetraphylla* was evaluated for antipsychotic activity on female Swiss albino mice. The antipsychotic activity was evaluated against and serotogenic (5-HT2A) and dopaminergic (DA-D2) receptors in-vitro and amphetamine induced hyperactive mouse model in-vivo. Additional toxicity and safety evaluation studies of methanol extracts of *R. tetraphylla* leaves at different doses (10, 100, 300 and 2000 mg/kg) on female Swiss albino mice and showed that methanol extract is non toxic. The isolated alkaloids are able to serve as a promising lead structure for drug development of treating psychotic conditions in human [80]. Fruits of this plant are used to cure spleen disorders.
Serpentine

Isoreserpinine

Tetraphylline

Corynanthine

Yohimbine

α-Yohimbine

β-Yohimbine

ψ-Yohimbine

Fig. 2.2.6. Structures of various isolated phytocompounds from *R. tetraphylla*. 
2.3. Extraction

Extraction is the separation of medicinally active parts of plant using suitable solvents through standard methods. The extracts obtained from plants are moderately complex mixtures of metabolites, in semisolid state or liquid state or in dry powder form, which can be used for oral or external application.

The aim of adopting standardized extraction procedures for crude drugs is to achieve the therapeutically desired fractions and to remove unwanted stuff by treatment with a different solvent. The extract obtained, after standardization, used as medicinal agent as such in the type of fluid extracts or tinctures or further processed in any dosage form such as capsules and tablets. These products contain composite mixture of many medicinal plant metabolites, such as alkaloids, glycosides, lignans, terpenoids and flavonoids [81].

The common techniques of medicinal plant extraction include maceration, infusion percolation, digestion, decoction, hot continuous extraction (Soxhlet), aqueous-alcoholic extraction by fermentation, microwave-assisted extraction, counter-current extraction, supercritical fluid extraction, ultrasound extraction (Sonication), and phytonic extraction (with hydro fluorocarbon solvents). Hydro-distillation techniques (water and steam distillation, water distillation and steam distillation), hydrolytic maceration followed by distillation [81].

In our present study we performed four types of extraction techniques as follows.

2.3.1. Maceration technique

In this technique coarsely powdered plant-drug is soaked in contact with the solvent in a stoppered container for a distinct period with repeated agitation until soluble matter is dissolved. Not much yield was obtained in this technique.
2.3.2. Infusion technique

In this technique a dilute solution of alcohol and water are used to extract from the plant material, but the obtained yield was less.

2.3.3. Sonication technique

In this technique involves the employ of ultrasonic frequencies at 50 kHz to agitate the particles of plant material, by this method not able to extract much yield.

2.3.4. Soxhlet extraction technique

A soxhlet extractor is a piece of laboratory apparatus invented in 1879 by Franz von Soxhlet [82]. It was originally designed for the extraction of constituents from a solid material. Typically, a soxhlet extraction is required where the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent. It is a continuous extraction of percolation type under hot condition. The active constituents of the plants are not freely soluble in the solvent or difficult to be displaced from the cells of the plant, then it become necessary to extract the crude drug by the action of hot menstrum for a considerable length of time. The apparatus used for continuous hot percolation process is soxhlet apparatus showed in the fig.2.3.1 which consist of three parts

![Fig.2.3.1. Soxhlet extractor used to extract medicinal plant materials](image-url)
Flask containing the boiling solvent.

Soxhlet extractor in which the drug to be extracted is packed.

A condenser in which the vapours of the solvent are condensed again into solvent

The soxhlet extractor was carried out with the solvents of varying polarity starting

- n-hexane
- Dichloromethane (DCM)
- Methanol (MeOH).

**Procedure**

Weighed amount (500 gm) of coarsely powdered plant material containing some of the desired compound is placed inside thimble made from thick filter paper, which is loaded into the main chamber of soxhlet extractor. The soxhlet extractor is placed on to a flask containing the extraction solvent. The soxhlet is then equipped with a condenser. Then solvent is heated to reflux. The solvent vapours travels up a distillation arm, and floods into the chamber housing the thimble of solid. The condenser ensures that any solvent vapour cools, and drips back down into the chamber housing the solid material. The chamber containing the solid material slowly fills with warm solvent. When the soxhlet chamber is almost full, the chamber is automatically emptied by a siphon side arm, with the solvent running back down to the distillation flask. Each extraction was carried out nearly for 18 hr. After each extraction, the remaining plant material was removed from extractor, dried and reloaded in the extractor for subsequent extraction until the solvent became colorless. Plant material was successively extracted using soxhlet extraction method. The extracts obtained were further concentrated by evaporating solvent using Buchi type evaporator under reduced pressure and controlled temperature. The extracts obtained were dried under vacuum, calculated the yield Table 2.3.2 to 2.3.4 and stored under
optimal condition for further use.

**Table. 2.3.1**: Name of the plant part subjected to extraction process

<table>
<thead>
<tr>
<th>Sl.No.</th>
<th>Name of the Plant</th>
<th>Part used</th>
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<tbody>
<tr>
<td>1</td>
<td><em>Alpinia Purpurata</em></td>
<td>Whole plant</td>
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<tr>
<td>2</td>
<td><em>Mimusops elengi</em></td>
<td>Leaves</td>
</tr>
<tr>
<td>3</td>
<td><em>Rauvolfia tetraphylla</em></td>
<td>Leaves and seeds</td>
</tr>
</tbody>
</table>

**Table. 2.3.2**: Different solvents used for soxhlet extraction and yield of crude extracts *Alpinia Purpurata*.

<table>
<thead>
<tr>
<th>Sl.No</th>
<th>Parts used</th>
<th>Solvent used</th>
<th>Colour and nature</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Whole Plant (500 gm)</td>
<td>n-Hexane</td>
<td>Green paste</td>
<td>27.58 gm</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>Dichloromethane</td>
<td>Green powder</td>
<td>28.34 gm</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>Methanol</td>
<td>Brown paste</td>
<td>34.21 gm</td>
</tr>
</tbody>
</table>

**Table. 2.3.3**: Different solvents used for soxhlet extraction and yield of crude extracts *Mimusops elengi*.

<table>
<thead>
<tr>
<th>Sl.No</th>
<th>Parts used</th>
<th>Solvent used</th>
<th>Colour and nature</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Leaves (500 gm)</td>
<td>n-Hexane</td>
<td>Green paste</td>
<td>24.58 gm</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>Dichloromethane</td>
<td>Green powder</td>
<td>25.25 gm</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>Methanol</td>
<td>Brown paste</td>
<td>28.24 gm</td>
</tr>
</tbody>
</table>

**Table. 2.3.4**: Different solvents used for soxhlet extraction and yield of crude extracts *Rauvolfia tetraphylla*.

<table>
<thead>
<tr>
<th>Sl.No</th>
<th>Parts used</th>
<th>Solvent used</th>
<th>Colour and nature</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Leaves (500 gm)</td>
<td>n-Hexane</td>
<td>Green paste</td>
<td>28.18 gm</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>Dichloromethane</td>
<td>Green powder</td>
<td>29.74 gm</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>Methanol</td>
<td>Brown paste</td>
<td>32.27 gm</td>
</tr>
<tr>
<td>4</td>
<td>Seeds (250 gm)</td>
<td>Methanol</td>
<td>Brown paste</td>
<td>10.86 gm</td>
</tr>
</tbody>
</table>
2.4. Phytochemistry

Phytochemistry is the study of phytochemicals, which are chemicals derived from plants. Exclusively, phytochemistry illustrates about the huge number of secondary metabolic compounds originate in plants. They also show a number of protective functions for human consumers [81]. The precise classification of phytochemicals could have not been performed up to now, because of the broad variety of them. In resent time Phytochemicals are classified as primary or secondary constituents, depending on their role in plant metabolism. Primary constituents include the common sugars, proteins, amino acids, pyrimidines and purines and of nucleic acids, chlorophyll’s etc. Secondary constituents are the remaining plant chemicals such as flavonoids, alkaloids, terpenes, lignans, plant steroids, curcumines, saponins, phenolics, and glycosides.

2.4.1. Some classes of phytochemicals

Alkaloids

Alkaloids are the major group of secondary chemical constituents made largely of ammonia compounds including fundamentally of nitrogen bases synthesized from amino acid building blocks with a variety of radicals replacing one or more of the atoms (hydrogen atoms) in the peptide ring, the majority containing oxygen. Most alkaloids are freely soluble in alcohol and though they are sparingly soluble in water. These nitrogenous compounds function in the defense of plants against pathogens and herbivores, and are extensively exploited as pharmaceuticals, narcotics and stimulants due to their resourceful biological activities. The name alkaloid ends with the suffix –ine and plant-derived alkaloids in clinical use include the analgesics morphine and codeine, the anticancer agent vinblastine, the antibiotics sanguinafine and berberine, the pupil dilator atropine, the antiarrythmic ajmaline, and the sedative scopolamine.
Other important alkaloids of plant origin include the addictive stimulants, nicotine, caffeine, codeine, atropine, morphine, ergotamine, cocaine and ephedrine (Fig.2.4.1).

![Morphine](image1.png)

![Codeine](image2.png)

![Caffeine](image3.png)

![Atropine](image4.png)

Fig.2.4.1. Structures of some significant plant derived alkaloids.

**Flavonoids**

Flavonoids are significant group of polyphenols broadly distributed among the plants. Basically, they are made of superfluous than one benzene ring in its structure (a range of C15 aromatic compounds) and lots of reports supports their employ as free radical scavengers or antioxidants [83]. The compounds are derived from parent compounds known as flavans. More than four thousand flavonoids are known to exist and some of them are pigments in higher plants. Quercetin and kaempferol are general flavonoids present in almost 70% of plants.

![Kaempferol](image5.png)

![Quercetin](image6.png)

Fig.2.4.2. Structures of some significant plant derived flavonoids.

**Phenolics**

Polyphenolics (or polyphenols extracts), Phenolics or phenols are class of chemical
compounds consisting of hydroxyl functional group (OH) attached directly to a benzene ring. Phenols are produced by plants, also acts as natural colour pigments which are responsible for the colour of fruits of plants.

Caffeic acid

Rutin

Resveratrol

**Fig. 2.4.3. Structures of some significant plant derived phenolics**

**Tannins**

They are polyphenolic compounds with high molecular weight. These are extensively scattered in many plant flora. Tannins form complexes with carbohydrates, proteins and alkaloids. The tannin compounds are extensively distributed in many species of plants, where they play a role in protection from predation.

Ellagic acid

Gallic acid

**Fig. 2.4.4. Structures of some significant plant derived tannins.**

**Terpenoids**

These are large and various class of naturally occurring organic compounds resemble to Terpenes, terpenoids are multi-cyclic structures that different from one another not only in functional groups but also in their primary carbon skeletons. About 60% of
identified natural products are terpenoids.

Fig. 2.4.5. Structures of some significant plant derived terpenoids.
2.4.2. Phytochemical analysis of crude extracts of medicinal plants.

The extracts so obtained were subjected to tests for the identification of different phytoconstituents like alkaloids, carbohydrates, triterpenoids, flavonoids, glycosides, proteins, steroids, saponins and tannins by standard methods [84]. To know that which classes of compounds are present in different solvent extracts.

1) Test for alkaloid

Mayer’s test (potassium mercuric iodide):
Mayers’s reagent was added to the acidic test solution (small quantity of extract + 2ml of water+ 2ml of Conc. HCl) gives cream colored precipitate indicates presence of alkaloid.

2) Test for steroids

Salkowaski’s test
To the test solution (small quantity of extract + 2ml of chloroform) few drop of Conc. sulfuric acid is added, shake well and then allow standing. The two layer turns red colour which indicates steroids are present.

3) Test for triterpenoids

Liebermann - Burchard’s test
2 mg of extract was dissolved in acetic anhydride, heated to boiling, cooled and then 1 ml of Conc. sulphuric acid was added along the sides of the test tube. Development of a pink colour indicates the presence of triterpenoids.

4) Test for carbohydrate

Molisch’s test
Small quantity of extract in test tube, few drop of molisch’s reagent and 2ml of Conc. sulphuric acid was added slowly through the test tube. A purple ring is formed at the junction of the two liquid indicates the presence of carbohydrate.
5) Test for flavonoids

**Ferric chloride test**

Small quantity of extract + 2ml water with few drops of ferric chloride solution gives intense green color indicates flavonoids are present.

6) Test for tannins

**Ferric chloride test**

Test solution (small quantity of extract + 2ml of water) with few drops of ferric chloride solution gives dark red colour which indicates presence of tannins.

7) Test for proteins

**Xanthoproteintest**

Test solution (small quantity of extract + 2ml of water) is treating with Conc. nitric acid on boiling gives yellow precipitate which indicates the presence of proteins.

8) Test of glycosides

**Killer-Killani test**

Few drops of ferric chloride solution added to extract and mixed well then Conc. sulphuric acid is added slowly two layers are formed. The upper layer is bluish green colour lower layer is reddish brown colour it indicates the presence of glycosides.

9) Test for saponins

**Foam test:**

Small quantity of extract + 3-5ml of water shake well, leading to the formation of froth which is stable at least 15 minute which indicates the presence of saponins.
Table 2.4.2.1. Phytochemical examinations were carried out for all the extracts as per the standard methods.

<table>
<thead>
<tr>
<th>Sl.No</th>
<th>Plant Constituents</th>
<th>Alpinia purpurata</th>
<th>Minusops elengi</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n-hexane</td>
<td>DCM</td>
</tr>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Steroids</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Terpenoids</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Carbohydrates</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Tannins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Proteins</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Glycosides</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>Sponin</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>


Table 2.4.2.2. Phytochemical examinations were carried out for all the extracts as per the standard methods.

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Plant Constituents</th>
<th>Rauwolfia tetraphylla</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n-hexane</td>
</tr>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Steroids</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Terpenoids</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Carbohydrates</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Proteins</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>Sponin</td>
<td>+</td>
</tr>
</tbody>
</table>

Dichloromethane-DCM, methanol of fruits-Methanol (F) and Indications: + = Present and - = absent.

Phytochemical screening of different solvent crude extracts of all three medicinal plants (Alpinia purpurata, Minusops elengi and Rauwolfia tetraphylla) revealed that it was a greater source for assortment of phytoconstituents.
2.5. Antimicrobial activity

2.5.1. Introduction

Antimicrobial activities refer to the activity of a compound to kill or inhibit the growth of micro-organisms. Micro-organism may acts as bacteriocides (killing bacteria), viricides (killing viruses), algicides (killing algae), or fungicides (killing fungi). Antimicrobial drugs are the highest contribution of the 20th century to human therapeutics. Drugs of this class are different from all others in that they are designed to inhibit or kill the infecting organism and to have no or minimal effect on the recipient. The composition of biologically active compounds of medicinal plants differs widely depending on the soil type, plant species, and on their association with microbes [85]. These bioactive secondary metabolites produced by medicinal plants can also powerfully affect plant-associated microbial communities and their physiological functions [86,87]. Even though an enormous number of medicinal plants have been well-studied with respect to their phytochemical constitutes and pharmacological properties, their microbiome and the physiological interactions between host and microbes remain inadequately understood [88]. Thus, they play an important role in chemotherapy to treat various infectious diseases. The literature survey revealed that variety of plants exhibit antimicrobial activity, which may be attributed to the secondary metabolites present in them. Therefore, the current preliminary screening of crude extracts was designed to evaluate whether the selected three medicinal plants were potent or proficient in antimicrobial activity or not, outcome of this study will give idea for further work. The medicinal plants was carried out against three bacteria viz., Staphylococcus aureus (Gram-positive), Escherichia coli (Gram-negative) and Pseudomonas aeruginosa (Gram-negative) and three Fungi viz., Cladosporium oxysporum, Penicillin chrysogenum and Candida
albicans by using the cup plate method [89].

2.5.2. Antibacterial activity

Drug used

Amoxicillin was used as standard for antibacterial activity.

Micro organisms used:

In present investigation three bacteria viz., *Staphylococcus aureus* (NCIM 2127-Gram-positive), *Escherichia coli* (NCIM 2065-Gram-negative) and *Pseudomonas aeruginosa* (NCIM 2945 Gram-negative). These cultures were procured from National Collection of Industrial Micro organism (NCIM), Pune, India.

The following materials are used for media (Nutrient agar) preparation:

- Sterilized petridishes and pipettes, test tubes and beakers
- Nutrient agar
- Sterilized fine pointed forceps
- Sterile 6 mm borer
- Sterile inoculation loops
- Tuberculin syringes
- Purified water
- Dimethylformamide (DMF) as a control

Preparation of media

Nutrient agar (16 g) was dissolved in purified water (1000 ml). The solution was sterilized for 20 min at 120°C and 15 Lb pressure in an autoclave.

Sterilization of media and glassware

Nutrient agar taken in conical flasks were sterilized by autoclaving at 15 Lb pressure for about 20 min. Test tubes, cork borer, pipettes, petridishes and beakers were sterilized in hot air oven at 110 °C for one hour.
Preparation of sub-cultures

One day prior to the experiment, the micro organisms were inoculated into sterilized nutrient broth tubes and incubated at 37°C for 24 h. The organisms were sub-cultured into sterile nutrient broth.

Preparation of test sample and standard

The test samples prepared as follows

- 10 mg test samples were dissolved in DMF 10ml in sterile test tubes and labeled as (1mg/ml).
- 10 mg test samples were dissolved in DMF 20ml in sterile test tubes and labeled as (0.5mg/ml).
- 10 mg test samples were dissolved in DMF 40ml in sterile test tubes and labeled as (0.25mg/ml).
- The Amoxicillin standard (10mg) was dissolved in DMF (10ml) to obtain 1mg/ml of concentration.

Method: Cup plate method

This method depends on diffusion of an antibiotic form a cavity through the solidified agar layer in a pertridish to an extent such that growth of the added microorganism is prevented entirely in circular area or zone around the activity containing a solution of antibiotic. A previously liquefied medium was inoculated with appropriate quantity of suspension of microorganism between 40-50 °C and inoculated medium was poured into pertridishes to give a depth of 3 to 4 mm, ensuring that the layers of medium were uniform in thickness by placing dishes on a leveled surface. The plates were dried under laminar airflow maintaining sterile conditions, with the help of sterile cork borer three cups of 6 mm diameter each were punched and scooped out. The three well were numbered for different test sample concentrations (1, 0.5, and 0.25
mg/ml) and standard drug were introduced into the bored cups. The petridishes were left standing for 2 hr at room temperature as period of pre-incubation diffusion to minimize the effects of variation in timing among the applications of different solutions. The inoculated plates were subjected to incubation for 24 hr at 37°C, shown in fig 2.5.2.1. The Zone of inhibition (ZOI) measurements was expressed in millimeter (mm) shown in table 2.5.2.1.

![Antimicrobial activity of Rauvolfia tetraphylla methanol extracts](image)

**Fig. 2.5.2.1 Antimicrobial activity of *Rauvolfia tetraphylla* methanol extracts.**
Table. 2.5.2.1 Antibacterial activity of selected medicinal plants of different extracts.

<table>
<thead>
<tr>
<th>Plant Extracts</th>
<th>Conc. samples (mg/ml)</th>
<th>Zone of inhibition in mm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>PA</td>
</tr>
<tr>
<td>Standard (Amoxicillin)</td>
<td>1mg/ml</td>
<td>22</td>
</tr>
<tr>
<td>AP (n-hexane extract)</td>
<td>1mg/ml</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>0.5mg/ml</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>0.25mg/ml</td>
<td>08</td>
</tr>
<tr>
<td>AP (Dichloromethane extract)</td>
<td>1mg/ml</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>0.5mg/ml</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>0.25mg/ml</td>
<td>09</td>
</tr>
<tr>
<td>AP (Methanol extract)</td>
<td>1mg/ml</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>0.5mg/ml</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>0.25mg/ml</td>
<td>12</td>
</tr>
<tr>
<td>ME (n-hexane extract)</td>
<td>1mg/ml</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>0.5mg/ml</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>0.25mg/ml</td>
<td>11</td>
</tr>
<tr>
<td>ME (Dichloromethane extract)</td>
<td>1mg/ml</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>0.5mg/ml</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>0.25mg/ml</td>
<td>10</td>
</tr>
<tr>
<td>ME (Methanol extract)</td>
<td>1mg/ml</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>0.5mg/ml</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>0.25mg/ml</td>
<td>14</td>
</tr>
<tr>
<td>RT (n-hexane extract)</td>
<td>1mg/ml</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>0.5mg/ml</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>0.25mg/ml</td>
<td>11</td>
</tr>
<tr>
<td>RT (Dichloromethane extract)</td>
<td>1mg/ml</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>0.5mg/ml</td>
<td>09</td>
</tr>
<tr>
<td></td>
<td>0.25mg/ml</td>
<td>08</td>
</tr>
<tr>
<td>RT (Methanol extract)</td>
<td>1mg/ml</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>0.5mg/ml</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>0.25mg/ml</td>
<td>14</td>
</tr>
<tr>
<td>RT (Seed methanol extract)</td>
<td>1mg/ml</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>0.5mg/ml</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>0.25mg/ml</td>
<td>12</td>
</tr>
</tbody>
</table>

P. A: Pseudomonas aureginosa, S. A: Staphylococcus aureus and E.coli: Escherichia coli,
Cork borer size 6mm.
2.5.3. Antifungal Activity:

Drug used

Flucanazole was used as standard for antifungal activity.

Micro organisms used:

In present investigation three Fungi viz., *Cladosporium oxysporum* (NCIM 1082), *Penicillium chrysogenum* (NCIM 1333), and *Candida albicans* (NCIM 3102). These cultures were procured from National Collection of Industrial Microorganism(NCIM), Pune, India.

The following material are used for media preparation

- Potato Dextrose Agar (PDA)
- Sterilized petri dishes and pipettes
- Sterilized test tubes and beakers
- Sterilized fine pointed forceps
- Sterile 6 mm borer and inoculation loops
- Potato Dextrose broth (48 hrs old)

Preparation of media

Potato Dextrose agar (PDA) (20 g) was dissolved in distilled water (500 ml). The solution was sterilized for 20 min at 121°C and 15 Lb pressure in an autoclave.

Sterilization of media and glassware

Potato Dextrose agar taken in conical flasks were sterilized by autoclaving at 15 Lb pressure for about 20 min. test tubes, cork borer, petri dishes, pipettes and beakers were sterilized in hot air oven at 110 °C for one hour.

Preparation of test sample and standard

For sample preparation refer antibacterial activity sample preparation.

The Flucanazole standard (10 mg) was dissolved in DMF (10 ml) to obtain 1mg/ml of
concentration.

**Preparation of sub-cultures**

Two day prior to the experiment, the micro organisms were inoculated into sterilized Potato Dextrose agar PDA broth tubes and incubated at 25°C for 48 h. The organisms were sub-cultured into sterile Potato Dextrose agar PDA broth.

**Method: Cup plate method or Zone of inhibition**

The method used during antibacterial testing was followed for antifungal testing. The test plates in case of antifungal testing were incubated for 48 hrs at 25°C shown in fig: 2.5.3.1. The Zone of inhibition (ZOI) measurements were expressed in millimeter (mm) shown in Table. 2.5.3.1.

![Image of fungal activity](image)

*Fig. 2.5.3.1. Anti fungal Activity of methanol *Alpinia purpurata* extracts.*
Table 2.5.3.1. Antifungal activity results of selected medicinal plants different extracts

<table>
<thead>
<tr>
<th>Name of Compounds</th>
<th>Concentration of extracts (mg/ml)</th>
<th>Zone of inhibition in mm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>PC</td>
</tr>
<tr>
<td><strong>Standard Flucanazole</strong></td>
<td>1mg/ml</td>
<td>23</td>
</tr>
<tr>
<td><strong>AP</strong> (Methanol Extract)</td>
<td>1mg/ml</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>0.5mg/ml</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>0.25mg/ml</td>
<td>14</td>
</tr>
<tr>
<td><strong>AP</strong> (n-hexane Extract)</td>
<td>1mg/ml</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>0.5mg/ml</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>0.25mg/ml</td>
<td>08</td>
</tr>
<tr>
<td><strong>AP</strong> (Dichloromethane extract)</td>
<td>1mg/ml</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>0.5mg/ml</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>0.25mg/ml</td>
<td>10</td>
</tr>
<tr>
<td><strong>ME</strong> (Methanol Extract)</td>
<td>1mg/ml</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>0.5mg/ml</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>0.25mg/ml</td>
<td>14</td>
</tr>
<tr>
<td><strong>ME</strong> (n-hexane Extract)</td>
<td>1mg/ml</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>0.5mg/ml</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>0.25mg/ml</td>
<td>09</td>
</tr>
<tr>
<td><strong>ME</strong> (Dichloromethane extract)</td>
<td>1mg/ml</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>0.5mg/ml</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>0.25mg/ml</td>
<td>08</td>
</tr>
<tr>
<td><strong>RT</strong> (n-hexane Extract)</td>
<td>1mg/ml</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>0.5mg/ml</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>0.25mg/ml</td>
<td>08</td>
</tr>
<tr>
<td><strong>RT</strong> (Dichloromethane extract)</td>
<td>1mg/ml</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>0.5mg/ml</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>0.25mg/ml</td>
<td>08</td>
</tr>
<tr>
<td><strong>RT</strong> (Methanol Extract)</td>
<td>1mg/ml</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>0.5mg/ml</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>0.25mg/ml</td>
<td>15</td>
</tr>
<tr>
<td><strong>RT</strong> (Seed Methanol extract)</td>
<td>1mg/ml</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>0.5mg/ml</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>0.25mg/ml</td>
<td>11</td>
</tr>
</tbody>
</table>

P. C: *Penicillium chrysogenum*, C. A: *Candida albicans* and C. O: *Cladosporium oxysporum*,
Cork borer size 6mm.
2.5.4. Results and discussions

The different plant extracts have potent medicinal value so they gave promising pharmacological action for various disease causing micro organisms, in this present study we analyzed the all three medicinal plant crude extracts against different pathogenic micro-organisms both bacteria as well as fungus, from the results we observed that the *Rauvolfia tetraphylla* (R.T) methanol extracts and *Mimusops elengi* (M.E) methanol extracts showed significant antimicrobial activity. The bacterial results indicated that the *R. tetraphylla* (R.T) and *M.elengi* (M.E) methanol was found to be active against gram positive bacteria (*P. aeruginosa*) and gram negative bacteria (*E.coli*), activity when compared with the standard Amoxicillin. The antifungal results shows that the *A. purpurata* (A.P) and *R. tetraphylla* (R.T) methanol extracts showed significant anti fungal activity when compared with the standard Flucanazole. These above significant crude extracts may be used in traditional medications to treat variety of infections caused by preferred microorganisms.
2.6. References.


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