2. REVIEW OF LITERATURE

In India, over 150 plants of various families reported to have hypoglycemic activity (Verma et al., 2011). Among them, *Clausena anisata* (Willd.) Hook f. ex. Benth, which is a medicinal plant belonging to the family *Rutaceae*, (Bhushan et al., 2009) is represented by 20 species in India and used traditionally for the treatment of several ailments such as rheumatism, cold, arthritis, analgesic, heart disorder, hepatic disease, antiseptic, anthelmintic, gastro intestinal disorders, fever, headache, sinusitis, wounds, sprains, fractures, toothache, mouth infection, stimulate insulin secretion (Hemalatha et al., 2012) and convulsions (Hutchings et al., 1996).

Eventhough, plants have been validated for its antidiabetic properties and its complications, there is a requirement to identify its phytoconstituents, its target, mode of action and treatment using plant products either alone or in combination with synthetic drugs (Noor et al., 2013).

2.1. Traditional and medicinal uses of *C. anisata*

Traditional medicine practitioners in Africa use the dried leaves of *C. anisata* like filling material for mattresses and pillows against lice, fleas and bedbugs. Roots are chewed to combat indigestion (Jain and Srivastava, 2005). As snake-bite antidote, to cleanse the uterus, in skin diseases (Ajibesin et al., 2008) and increase milk production after child birth (Schmelzer and Gurib, 2013), gonorrhea and haemorrhoids (Ajibesin et al., 2008), as pesticides (Ajibesin et al., 2008), antifeedant in worms (Mukandiwa et al., 2013), anti-inflammatory (Balde et al., 2015), antifungal and antiviral (Hamza et al., 2006; Moshi et al., 2009), antibacterial activity (Venkatesalu and
Senthilkumar, 2009; Afolayan et al., 2015), diabetes (Ojewole, 2002; Mogale et al., 2012), rheumatism, migraine headache (Rupande and Bukaliya, 2013), management of epilepsy (Kenechukwu et al., 2012), cough and treatment of tuberculosis (Yineger and Yewhalaw, 2007), mosquito repellant against Anopheles arabiensis (Maharaj et al., 2010; Mavundza et al., 2014; Arbab et al., 2012; Jain and Srivastava, 2005), syphilis, kidney ailments and against HIV (Rupande and Bukaliya, 2013).

The leaf and root decoction were taken for gastrointestinal disorders, pneumonia (Schmelzer and Gurib, 2013), antiplasmodial and analgesic (headache, toothache) (Okoton et al., 2012) and antipyretic, hypotension, venereal diseases (Schmelzer and Gurib, 2013), sore throat and sinusitis, as an aphrodisiac (Schmelzer and Gurib, 2013), and anthelmintic (Miaron et al., 2006), impotence and sterility, taeniasis, schistosomiasis (Sahu et al., 2015), mental illness and schizophrenia (Prashant et al., 2014), antioxidant and crushed leaves were used in treating wounds (Agyare et al., 2015), as a tonic to cure breast pain in pregnant women and as a tonic to prevent rickets (Kadiri et al., 2015) and control convulsions in infants (Schmelzer and Gurib, 2013).

A mixture of C. anisata, Afraegle paniculata and Azadirachtha indica (called “Agbo”) was taken against gut disturbance and malaria in Nigeria. In South Africa leaves, were applied against high blood pressure (Arbab et al., 2012).

A decoction prepared from boiled stem bark extract of C. anisata was taken internally for two weeks by akwa ibom state people to treat measles (Ajibesin et al., 2008). The mature air dried rhizome was added to cold water and boiled under reduced temperature for 45 minutes for treating lung
ulceration in the management of TB in Eastern Cape Province (Afolayan et al., 2014; Gizachew et al., 2013). Due to its pungent and aromatic smelling it was used in stress related disorders (Hutchings, 1989). Several studies have been conducted to comprehend these effects.

2.2. Phytochemistry of C. anisata

The major phytoconstituents that are present in this plant are:

2.2.1. Alkaloids: The major constituent of this plant was found to be carbazole alkaloid, which belongs to the class 1-oxygenated-3-methoxy-carbazole that has a prenyl side chain or an analogues moiety at C4. The alcoholic stem bark extract contains clausenol and clausenine. The combined root and stem bark extracts was found to have 1-methyl-3, 4-dimethoxy-2-quinolone and 3-formyl-1-hydroxycarbazole and four known alkaloids were heptaphylline, girinimbine, ekebergnine and 3-methylcarbazole (Ito et al., 2009). Another study conducted by Ito et al., (2000) on root extract led to the isolation of four new carbazole alkaloids named clausamine D, E, F and G. Ito et al., (2009) and Arbab et al., (2012) isolated and identified eight known and two new carbazole alkaloid named furanoclausamines A and B from stem of C. anisata collected from Thailand. In another study Connolly et al., (1989) anisocoumarins A, B, C and D were also isolated from the combined stem bark and root extracts of C. anisata.

White et al., (2012) reported that the root and stem bark of C. anisata possess compounds like girinimbine, murrayamine-A and ekebergnine; two peptide derivatives like aurantiamide acetate and N-benzoyl-L-phenylalaninyl-N-benzoyl-L-phenylalaninate and a mixture of two phytosterols: sitosterol and stigmasterol that were isolated and the structure was established by H-NMR, C-NMR, COSY, HSQC, HMQC, HMBC, NOESY and MS.
2.2.2. **Coumarins:** The reinvestigation of *C. anisata* leaf extract by Arbab *et al.*, (2012) yielded imperatorin, xanthotoxol, lansamide-1 and three new furanocoumarins namely indicolactone, anisolactone and 2’, 3’ – epoxyanisolactone, anisocoumarins I and J. The structures of the compound were elucidated by spectroscopic and chemical methods.

Several studies have been reported for the isolation of new compounds from *C. anisata*. The leaves stem bark and roots led to the isolation of twenty one coumarins. Out of which Ngadjui *et al.*, (1991) isolated two new geranyl coumarins – anisocoumarins I and J from the leaves and compound was identified based on the spectral data and chemical correlation.

2.2.3. **Phenylpropanoids:** The essential oil obtained from hydrodistilled leaves of *C. anisata* showed anethole (31.1%), trans-β-ocimene (20%), β-elemene (10.5%), sabinene (33%), germacrene –D (17%), Z- β- ocimene (6%), germacrene –B (5.5%), (E)- β- ocimene (4.9%), terpinen-4-ol (4.7%), estragole (6.9%), α-pinene (6.7%) and γ-cadinene (5.4%) (Venkatesalu and Senthilkumar, 2009; Arbab *et al.*, 2012; Usman *et al.*, 2010).

2.3. **Ethnopharmacological relevance and validation of *C. anisata***

A study has been conducted by Mukandiwa *et al.*, (2013) to comprehend the ethnoveterinary medicine of *C. anisata* to expel maggots from wounds in animals. It was observed that the compound seselin, chemically known as 2’2’-dimethylpyranocoumarin was isolated from n-hexane fraction from acetone extract leaf powder, inhibited the feed intake of blowfly larvae in the first and second instars at a minimum concentration of 1ppm.
Teklehaymanot and Giday, (2007) reported that *C. anisata* leaf juice was used as an ear drop and its roots were chewed for stomachache by local people in Africa. The fidelity level (FL) of this plant was found to be 100%.

The aqueous cream of the ethanolic leaf extract was evaluated by Agyare *et al.*, (2015) in excision wound model of Sprague-Dawley rats for its wound healing activity under *in vivo*, in a concentration of 7 and 10% w/w. The 7% w/w was found to significantly increase the rate of wound contraction at days 13 to 19 compared to untreated rats.

Mukandiwa *et al.*, (2012) tested the acetone and aqueous extracts of *Aloe zebrine, C. anisata, Erythrina lysistemon* and *Spirostachys africana* as a remedy for treatment of myiasis. This study revealed that the plant extract (10-150 mg/ml) may contain compounds that interfere with larval feeding and the neuroendocrine control mechanism in the blowfly. The increase in concentration of plant extracts of *C. anisata* and *S. africana* was found to be associated with the emerging adult flies being smaller.

Efferth *et al.*, (2014) investigated the methanolic extracts of eleven medicinal plants from Cameroonian *Beilschmiedia acuta* Kosterm, *C. anisata, Fagara tessmannii* Engl, *Newbouldia laevis* Seem and *Polyscias fulva* Harms against multifactorial drug resistance of human cancer cell lines. All extracts at a concentration of 40µg/ml inhibited more than 50% proliferation of CCRF-CEM cells.

Tatsimo *et al.*, (2015) monitored the leaves and stem bark extracts of *C. anisata* by LC-HR-MS analysis which lead to the isolation and characterization of twenty one secondary metabolites: Clausamine H (1), three carbazole alkaloids (2-4), fourteen coumarins (5-18), two porphyrin
derivatives (19-20) and one limonoid (21) that can be used as anticancer drug. The study showed compounds like murrayamine-A (4), 3-(1, 1-dimethyl allyl) xanthyletin (5), gravelliferone (7), excavatin D (10), 7-[(E)-7-hydroxy-3, 7-dimethylocta-2, 5-dienyloxy]-coumarin (13), phellopterin (15) and 1-O-methylclausenolide (21) were found to be active against HeLa cell with LC_50 ranging from 1.14 to 3.26µg/ml. All this compounds were highly potential to be used as anticancer drug, as it was non toxic to normal cells.

Yineger and Yewhalaw, (2007) reported that the fresh form of juice obtained from the leaves of *A. quartinianus, C. anisata, M. salicifolia, Myrsine africana* L. and *P. schimperi* was drunk in very small amount for three days to treat Naqarsaa (Cancer) by the people in Sekoru District, Jimma zone, Southwestern Ethiopia.

Itoigawa *et al.*, (1999) isolated the new carbazole alkaloids clausamine D, E, F and G that belongs to the class of 1-oxygenated 3-carbomethoxy carbazole having a prenyl moiety at C-4 from the acetone extract of dried branches of *C. anisata* using Si gel column and preparative thin layer chromatography, was found to have anti-tumor promoting activity against TPA-induced EBV-EA activation in Raji cells.

Emerole *et al.*, (1981) isolated furanocoumarins like imperatorin; oxypeucedanine and chalepin from *C. anisata* and were tested for its anticoagulant activity by administering to rats. Of which chalepin treatment possessed anticoagulant activity by increasing α-1-globulin and decreasing β-globulin content of the serum and very mild necrosis of hepatocytes was also reported in chalepin treated rats.
Okoton et al., (2012) tested crude leaf extract (39-117 mg/kg) and its fraction (chloroform, aqueous (78 mg/kg)) of *C. anisata* on mice infected with chloroquine-sensitive *Plasmodium berghei* using suppressive, prophylactic, curative models for its antimalarial and analgesic activity against acetic acid, formalin and heat induced pains. This study concluded that extract and its fractions dose dependently reduced parasitaemia and inhibited the inflammation mediated through its chemical constituents.

Okoton et al., (2012) found that the crude leaf extract (39-117 mg/kg) of *C. anisata* reduced pyrexia by reducing concentration of prostaglandin E₂ in brain through its action on COX-3 or by enhancing the production of the bodys own antipyretic substances like vasopressin and arginine especially in hypothalamus. Form this study it was observed that the extract inhibited DNP-, amphetamine and yeast induced pyrexia.

Eloff et al., (2015) studied the anti-inflammatory activity of 25 plant species extracts that was used traditionally to treat pain in South Africa. This study measured the inhibitory effect of extracts based on the activities of pro-inflammatory enzyme, lipoxygenase and inducible nitric oxide synthase. The results showed that the crude acetone extract of *C. anisata* had best inhibitory activity on NO production (96.9%) at 6.25 µg/ml.

Studies have proved the larvicidal activity of *C. anisata* against *A. aegypti* (Mukandiwa et al., 2015), acetone, dichloromethane, hexane crude leaf extracts and compounds were evaluated at a concentration of 12.5, 25, 50, 100 and 200 ppm. The results showed 90% mortality at 100 ppm. Only the hexane fraction containing the compound seselin was found to cause mosquito larval mortality against *A. aegypti*. This larvicidal activity was found to retain even after two months of storage.
Venkatesalu et al., (2015) obtained the hexane, chloroform, ethylacetate, acetone and methanol extracts from seven aromatic plants viz., *Blumea mollis*, *Chloroxylon swietenia*, *C. anisata*, *Feronia limnonia*, *Lantana camera*, *Plectranthus amboinicus* and *Tagetes erecta* and screened against *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi* for its larvicidal activity. The larvicidal activity of these plant extract was found to be in the order of ethyl acetate extract of *C. anisata* > methanol extract of *Plectranthus amboinicus* > acetone extract of *Feronia limnonia* > methanol extract of *Tagetes erecta* > methanol extract of *Blumea mollis* > methanol extract of *Lantana camera*. The ethylacetate extract of *Chloroxylon swietenia* was found to be most potent against the larvae.

The larvicidal activity of leaf essential oil of *C. anisata* obtained by hydro-distillation was studied by Sritabutra and Soonwera, (2013) against *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi*. The GC-MS analysis of this study showed β-pinene (32.8%), sabinene (28.3%), germacrene-D (12.7%), estragole (6.4%) and linalool (5.9%) as a major chemical constituent in the essential oil and revealed that leaf essential oil can be used as potent natural larvicides.

The antimalarial activity of the chloroform and hexane extracts was tested against *Plasmodium berghei* ANKA strain, in which 500 mg/kg/day of the chloroform extract was found to exhibit 66.1% and 73.4% parasite reduction in the prophylactic and suppressive tests respectively (Irungu et al., 2012).

The fidelity level and consensus factor (ICF) for sixty seven ethnomedicinal plant species used by traditional healers to manage 51
different human ailments were identified and documented by Yineger et al., (2008). This study showed a fidelity level and ICF of *C. anisata* for the treatment of rheumatism to be 33.33% and 0.29 respectively.

From the alcoholic stem bark extract of *C. anisata*, two new carbazole alkaloid (clausenol and clausenine) was isolated by Chowdhury et al., (1995). The physical, chemical evidence and synthesis established their structures as 1-hydroxy-6-methoxy-3-methylcarbazole and 1, 6-dimethyl-3-methyl carbazole respectively, in which clausenol was found to be active against gram positive bacteria, gram negative bacteria (Venkatesalu and Senthilkumar, 2009) and fungi.

A study carried out by Afolayan et al., (2015) confirmed the antibacterial activity of *C. anisata* leaves. Of the different extracts (acetone, water and dichloromethane), the acetone leaf extract showed potent antibacterial activity with MIC ranging from 0.1 to 5mg/ml for *S. pyogens*, *S. aureus*, *B. cereus*, *E. faecalis*, *Listeria monocytogenes*, *S. typhimurium*, *Serratia marcescens*, *P. aeruginosa*, *E. coli* and *Shigella flexnerii*.

*C. anisata* has been used by traditional healers for the treatment of oral candidiasis and fungal infections of the skin. Hamza et al., (2006) validated the strong antifungal activity exhibited by the crude methanolic extracts of *C. anisata*, *Sclerocariya birrea*, *Turraea holstii*, *Sterculia africana* Fiori, *Acacia robusta* subsp. *Usambarensis* (Taub) Brenan, *Cyphosterma hildebrandti* (Gilg) Desc, *Elaeodendron buchannanii* (Lows), *Acacia nilotica* (L.) Wild ex Del, *Jatropha multifida* L., and *Pteridium aquilinum* (L.) Kuhn by using broth microdilution method. The most susceptible yeasts was found to be *Cryptococcus neoformans*, followed by *Candida krusei*, *Candida tropicalis* and *Candida parapsilosis*. The least susceptible were *Candida albicans* and *Candida glabrata*. 
The essential oil from the three chemo-varieties of *C. anisata* leaves namely estragole, trans anethole (74.3-89.6%) and feniculin were screened by Meijer, (1947) against six bacteria (*E. coli*, *S. aureus*, *S. typhi*, *Shigella sp*, *Proteus sp* and *Pseudomonas aeruginosa*) and three fungi (*Candida albicans*, *Aspergillus niger* and *Aspergillus parasiticus*) using disc sensitivity test (Osei-Safo et al., 2010). The result showed that the estragole rich oil exhibited significant activity against *E. coli* (16.3±0.3) and *Shigella sp* (17.2±0.4 mm). The trans anethole rich oil exhibited less significant activity (11.4±0.7 mm and 12.1±0.3 mm respectively), whereas the feniculin rich oil alone and in combination with the trans anethole rich oil did not showed any significant activity against the microbes tested.

Tan and Nishida (2012) reviewed and discussed the occurrence and distribution of methyl eugenol in different plant species. In leaf essential oil of *C. anisata* nearly 90% of methyl eugenol was found that act as an antifungal, antibacterial (Gundidza et al., 1994), antinematodal, toxicant roles against pathogens and insect herbivores. Methyl eugenol also functions as an antifeedant especially in tephritid fruit flies.

Eloff et al., (2012) and Eloff et al., (2014) examined the acetone leaf extracts of thirteen plant species in South Africa which was used to treat gastrointestinal helminth infection for antifungal activity by serial microdilution with tetrazolium violet as growth indicator against *Aspergillus fumigatus*, *Cryptococcus neoformans* and *Candida albicans* These pathogens caused an opportunistic infections in immune compromised patients and the study showed *C. anisata* had a good antifungal activity with MIC value as low as 0.02mg/ml against *Aspergillus fumigatus*. Its lowest cytotoxicity (LC$_{50}$) of 0.17mg/ml was found to have a therapeutic index (2.65) against *A. fumigatus*. 

The ethanolic extract of *C. anisata* was analysed for its antimicrobial activity against Gram positive bacteria (*Bacillus subtilis*, *S. aureus*, *Enterococcus faecalis*, *Bacillus thuringiensis*), Gram negative bacteria (*P. aeruginosa* and *P. vulgaris*) and a clinical isolate of *C. albicans* using agar well diffusion and micro dilution methods (Agyare *et al.*, 2014). The study showed MIC ranging from 0.5 to 0.7 mg/ml, 2.5 to 1 mg/ml and 5.5 mg/ml was active against gram positive, gram negative and *C. albicans* respectively and also phytochemical screening analysis of the plant showed the presence of tannins, flavonoids, steroids, saponins, glycosides and alkaloids.

Pawar *et al.*, (2011) found that the chemical constituent like sabinene, germacrene D in *C. anisata* possess antibacterial and antifungal activity against *Flavobacterium suaveolens*, *Serratia marcescens*, *Alcaligenes faecalis*, *Geotrichum candidum*, *Aspergillus parasitic*, *P. citinum* and *Alternaria alternate*.

Yaouba *et al.*, (2011) extracted chemical constituents like E-ocimenone, (15.1%), Z-ocimenone (11.5%), gamma-terpinene (11.4%) and germacrene D (10.9%) from the essential oil from fresh leaves of *C. anisata* and analysed for its antifungal and antiradical scavenging activities. This study indicated the inhibition of *A. flavus*, *A. niger*, *A. parasiticus* and *F. moniliforme* at a concentration of 4, 5, 5 and 5 mg/ml of *C. anisata* essential oil. The antiradical scavenging activity was found to be SC50= 5.1g/L.

Loon *et al.*, (2004) and Boeke *et al.*, (2004) evaluated the toxic and anti repellent activity of *C. anisata*, *Dracaena arborea*, *T. vogelii*, *Momordica charantia* and *Blumea aurtia* powders against the beetle *Callosobruchus maculates*. In this study the repellence was evaluated by observing the behavior of female beetles exposed to treated and untreated cowpea in a linear olfactometer. The plant powders were found to be repellent to the beetle.
The leaf powder of *C. anisata* and *Plectranthus glandulosus* was evaluated against Cameroonian and german strains of *Sitophilus zeamais* and *Prostephanus truncates* for their insecticidal activities, effects on progeny production, grain damage and population increase (Nukenine *et al.*, 2010; Goudoum *et al.*, 2013). The significant mortality of the three insects was found, in which *P. glandulosus* found to be more potent than *C. anisata*.

The insecticidal activities and their effects on progeny production of *Acanthoscelides obtectus* on essential oil extracted from *C. anisata* and aromatized clay powder was assessed by Tapondjou *et al.*, (2008). The results showed that moderate repellent activity in essential oil and high fumigant toxicity (*LC*<sub>50</sub>=0.093 µl/cm<sup>3</sup>) against *A. obtectus* and clay powder was used to stabilize the essential oil to increase its insecticidal efficacy. The compound responsible for antifeedant activity in root extracts of *C. anisata* was found to be osthol, 7-hydroxy coumarin derivative (Wang *et al.*, 2009).

Eloff *et al.*, (2013) carried out a study to influence the egg hatching and larval development of *Haemonchus contortus*. The results showed the acetone extract of *C. anisata* had least toxic with an *LC*<sub>50</sub> of 0.17 mg/ml and with moderate egg hatching activity with *EC*<sub>50</sub> values ranging between 1.48 – 5.70 mg/ml.

Muthee *et al.*, (2011) documented the utilization of anthelmintic plants for the management in animals and humans by the people of Loitoktok district in Kenya. The most frequently used anthelmintic plants were *Albizia anthelmintica*, *Myrsine africana*, *Rapanea melanophleos*, *Clausena anisata* and *Olea africana* used by 70%, 70%, 26%, 13% and 9% of the respondents respectively. To validate, this study was conducted by Eloff *et al.*, (2014) which showed that the acetone leaf extract of *C. anisata* had most promising anthelmintic activity.
Sohounhloue *et al.*, (2004) studied the volatile oil obtained from the leaves of *C. anisata* by hydrodistillation using GC and GC/MS to study the radical scavenging ability and antioxidant activity. The major constituent was found to be methyl-chavicol like estragole (66.2%), and (E)-anethole (17.6%). In this study the activity of the extract was found to be very close compared to the reference compound.

A study conducted by Chan *et al.*, (2014) on root extracts of *C. lansium* led to the isolation of eight new carbazole alkaloids like claulamines C(1), D(2) and E(5), clausenalines B-F (3, 4, 6-8), four new coumarins, clausemarins A-D (9-12) and 43 known compounds. Using 2D-NMR and ECD spectra the structure of new compounds and their absolute configuration were established. The results showed that the compounds 9, 13-18 and 20-22 exhibited strong inhibition of superoxide anion generation with IC$_{50}$ values ranged from 1.9 to 8.4µM; while compounds 18, 19 and 21 inhibited elastase release with IC$_{50}$ values 2 to 6.9 µM.

The experimental study of Staden *et al.*, (2012) demonstrated that the freshly harvested materials and long term storage (12 or 16 years) materials of medicinal plants like *Artemisia afra*, *C. anisata*, *Cussonia spicata*, *Leonotis intermedia* and *Spirostachys africana* were compared for its phytochemical, antioxidant and acetylcholinesterase-inhibitory properties. The results showed that prolonged storage of materials under dark conditions at room temperature found to retain high phenolic and flavanoid content. The DPPH radical scavenging activity was higher for fresh material and acetylcholineesterase-inhibitory activity was found to be similar for both the materials. In another study the ethanolic leaf extract of *C. anisata* was found to possess antioxidant activity with IC$_{50}$ 32.9 µg/ml.
Ayisi and Nyadedzor, (2003) compared the extracts of *Ocimum gratissimum* (GHX-2), *Ficus polita* (GHX-6), *C. anisata* (GHX-7), *Alchornea cordifolia* (GHX-26) and *Elaeophorbia drupifera* (GHX-27) against HIV-1, HIV-2 replication and cytopathicity. The results showed that all extracts inhibited HIV-1 strain HTLVIII B cytopathicity. GHX-2 leaves and seeds of GHX-26 showed high antiviral indices. The EC<sub>50</sub> values ranged from 0.005 to 0.075 mg/ml for GHI against HIV-2, when the treatment was started at 40 mins after virus adsorption. The GHX-7 showed moderate activity and GHX-26 had no activity.

Ruangrungsi *et al.*, (2000) isolated the four new carbazole alkaloids (Causamine D (1), E (2), F (3) and G (4) from *C. anisata* as inhibitors for Epstein – Barr virus. This compound induced the activation of early antigen by 12-O-tetradecanoylphorbol-13-acetate in Raji cells.

Kenechukwu *et al.*, (2012) studied the antiepileptic activity against pentylenetetrazole (PTZ) induced seizures in mice using ethanolic root bark, stem bark and leaf extracts of *C. anisata*. The study showed that the root bark extract (800 mg/kg) was found to contain bioactive constituents which possess anticonvulsant activity that used to manage petitmal epilepsy.

The plant *C. anisata* are also used for the treatment of high blood pressure by local healers in Bugabo (Moshi *et al.*, 2009), known to have anticonvulsant activity (Adesina and Ette, 1982; Makanju, 1983) and angiotensin converting enzyme (ACE) inhibitory activity (Duncan *et al.*, 1999).

The leaf powder of *C. anisata* was mixed with water and given to the snake bite victim (Birhanu *et al.*, 2015), bark of *C. anisata*, leaves of *Sida rhombifolia*, root of *Cucumis ficifolius*, bark root of *Brucea antidysentrica* are powdered together and mixed in milk then drunk a cup of tea for three days in order to get cured from Rabies disease. Nine juvenile leaves of *Calpurnia*
*aurea*, nine leaves of *Senna occidentalis* and nine juvenile leaves of *C. anisata* are smashed and the extract was taken. This one cup of tea for adult and half cup for children was given to treat ascaris in Wayu Tuka District of Oromia Region, Ethiopia (Megersa *et al.*, 2013).

The saponins and terpenoids from *C. dentata* were found to be effective in degradading endosulfan, an organochlorine insecticide (Archaya *et al.*, 2015). From the results the significant degradation was found in saponins at a concentration of 1000 and 2000 µg/ml of endosulfan with 1 and 2 ml of secondary metabolites.

The crude aqueous and methanol extract of *C.anisata, O. laniifolium* and *M. africana* were tested for its antimycobacterial activity against *Mycobacterium tuberculosis* H37RV strain and *M. bovis* (SB1176) that showed MIC range of 400-1600 µg/ml for the extracts of three plant species (Gizachew *et al.*, 2013).

Adebajo *et al* (2009) studied antitrichomonal, anti-diabetic, anti-inflammatory, anti-hepatotoxic and antioxidant activities of stem bark extract of *C. lansium* to justify the ethnomedicinal uses. The isolated compounds imperatorin and 3-formylcarbazole from dichloromethane extract was found to have potent antitrichomonal activity. The methanolic extract was found to have maximum antihyperglycaemic activity by inducing insulin release in INS-1 cells compared to control. And also the extract possesses good anti-inflammatory and antioxidant activity.

Laphookhieo *et al.*, (2012) isolated and characterized two new carbazole alkaloid along with twelve known compounds from the acetone and hexane extracts from the roots of *C. lansium*. The isolated mafaicheenamine E exhibited cytotoxicity against MCF-7 cell line (IC$_{50}$ 3.1 µg/ml) that possesses to have anti cancer activity.
Coumarins like 3, 10-bis (1, 1-dimethyl prop-2-en-1-yl)-5, 6, 7-trihydroxy-8, 8-dimethyl-7, 8-dihydro pyranochromen-2-one, bergapten and xanthotoxin were isolated from the roots and stem bark of *C. pentaphylla* (Aslam and Intekhab, 2008).

A novel and antidementia compound clausenamide (-clau) was isolated from *C. lansium* by Zhang and Chu, (2014) and was found to be more promising for the treatment of Alzheimers disease. The compound –clau contains four chiral centers and yielded eight pairs of enantiomers.

### 2.4. Induction of Diabetes in rats

Alloxan an oxygenated pyrimidine derivative, hydrophilic, unstable chemical compound, present as aqueous solution in the form of alloxan hydrate. This drug is diabetogenic when administered intravenously, intra peritoneally and subcutaneously. Depending on the animal species, route of administration and nutritional status the dosage is selected. It is reported that alloxan is non-toxic to the human beta cells and hence it is used to induce experimental diabetes. The multiphasic changes such as transient hypoglycemic phase, hyperglycemic phase, hypoglycemic phase and diabetic hyperglycemic phase caused by alloxan are irreversible and cause necrotic cell death of pancreatic islets. Also the alloxan results in highly reactive hydroxyl radicals that are formed due to fenton reaction in the presence of ferrous and hydrogen peroxide. It increases cytosolic free calcium concentration in beta cells of islets that depolarize the beta cell membrane and opens VDCC that enhance calcium entry into pancreatic cells. This calcium ion causes damage of beta cells of pancreatic islets (Mohan *et al.*, 2014; Rohilla and Ali, 2012).
2.5. Role of SNPs in Herbal Nanotechnology

The properties and physical characters of nanomaterials are used to treat several diseases at molecular level. The size, distribution and morphology of nanoparticles of silver salts are being used in various research areas like pharmacy (anticancer, antidiabetic, antibacterial, epilepsy, gastroenteritis, syphilis and gonorrhea), shampoos, soaps, detergents, cosmetics, toothpaste and medicine (Das et al., 2014).

The inhibitory effect of silver towards many bacterial strains and microorganisms commonly present in medical and industrial processes. In skin ointments and cream contains silver or SNP to prevent infection of burns and open wounds and also in medical devices, implants and silver embedded fabrics in sporting equipment. Hence SNP is playing an important role in herbal nanotechnology to prevent chronic diseases like diabetes and cancer.

Silver is naturally occurring element, slightly harder than gold, ductile and malleable. Highest electrical, thermal conductivity and lowest contact resistance was found in pure silver (Ponarulselvam et al., 2012).

The different oxidation states of silver are Ag$^0$, Ag$^{2+}$ and Ag$^{3+}$. Of which Ag$^0$ and Ag$^{2+}$ are most abundant one; Ag$^{3+}$ is unstable in aquatic environment. Silver nitrate, silver chloride is soluble in water and metallic silver is insoluble in water. The toxicity of silver in the environment is dependent on the availability of free silver ions. The primary route of entry for silver compounds and colloidal silver proteins is ingestion. The dietary intake of silver is 70-90µg/day and any form of silver is not toxic, not carcinogenic to
immune, cardiovascular, nervous and reproductive system. The phytoconstituents (terpenoids, flavones, ketones, aldehydes, amides and carboxylic acids) in plants acts as a capping agent and reduce silver ions for nanoparticle synthesis as they are free from toxic chemicals (Ikram et al., 2016). Hence the nanoparticle synthesis using plant extract is the most adapted method compared to physical, biological and chemical methods. The major advantage of using plant extracts are easily available, safe and nontoxic (Kalakotla et al., 2015).

Due to smaller size, rapid entry into target cells, biodegradable nature, greater bioavailability of drug, lesser drug requirement and ability to cross blood brain barrier the nano-encapsulated drugs are more commonly used in herbal nanotechnology to cure several ailments (Ikram et al., 2016). It is also reported that when SNPs are released into nature (soil or water) they do not last longer as NPs, instead they grow as harmless clumps of silver metal. It is water insoluble, nontoxic, nonionic and hence it do not release silver ions into the environment, unless strong oxidizing agent is present (Maass, 2008).

The antimicrobial property of SNPs depends on i) size and environmental conditions such as pH, ionic strength, ii) capping agent. The Ag ions that possess positive charge is important for its anitimicrobial activity and it should be in ionized form. Because in ionized form, when it comes in contact with moisture it releases silver ions. Ag⁺ ions interact with the nucleosides and are able to form complex and thereby denature DNA, cause damage to cell wall and membrane (Ikram et al., 2016).
In order to reduce drug degradation, drug loss, to prevent harmful side effects and to increase drug bioavailability the use of plant extracts for synthesis of NPs become a new dimension. A new carrier which that possess site specific targeting and drug delivery is the metallic NPs. Due to good conductivity, chemically stable, catalytic activity, antimicrobial activity, increase in oral bioavailability and to overcome the poorly water soluble herbal medicines, the SNPs are used in various applications in medicine (Aruna et al., 2014).

Hence based on above findings and reports on various biological activities attributed by C. anisata, formed the basis of the study. Thus the present study was carried out to further analyse the crude and SNP extracts of this selected plant for its antioxidant, antibacterial and hypoglycemic activity under in vitro and in vivo conditions.