Chapter 7
Summary
&
Conclusion
7.0 SUMMARY AND CONCLUSION

7.1 Summary

In the current past the medication world has adjusted their complete focus on the conventional drugs and natural treatment because of the consequences and toxicity of the man-made counterpart is gradually more. Therefore the current work was carried chiefly focused on recognition of antimicrobial ingredients from the conventionally used medicinal plants. Plants are the vital source of facts about modern medicine. Comparatively lower numbers of unfavorable reactions to plant products as for modern conventional pharmaceuticals, together with their cheap cost, is encouraging equally the consuming national health care institutions and public to think plant medicines as an option to synthetic drugs. Currently herbal medicines are prescribed generally yet their biologically dynamic compounds are unidentified because of their efficiency and no consequence in clinical practice. Microorganism’s resistance to the majority of antibiotics is quickly spreading. As a result there is a critical requirement for new antibiotics. Nearly all antibiotics have been obtained from microbes. A plant signifies an appealing source for discovery of new antimicrobial compounds. Against the microorganisms they are finely protected, because of the existence of antimicrobial compounds or the initiation of phytoalexin biosynthesis following infection. This thesis spotlight mainly on some plentifully existing plant sources, with the suggestion that plants used before now in agricultural processing industry may be the cause of antimicrobial active ingredients which would add spare value to these harvests. For the current study medicinal plant *Ipomoea reniformis* was selected. The introduction of antibiotics transformed the way by which infectious sickness were treated. Rapidly, widespread infections became effortlessly curable and outbursts of infectious ailment were eagerly controlled. Though, the announcement of triumph over bacterial pathogens was early. Antimicrobial resistance rapidly emerged to decrease the clinical value of every novel antibiotic that was developed. Alleviation of antimicrobial resistance is therefore essential, and necessitates that veterinarians and further health proficient’s appreciate antibiotic sensitivity and resistance at the organism, population, molecular and cellular levels. The effort is intended for the antimicrobial action for (bacterial and fungal species) and separation and categorization by spectral analysis. Followed by antioxidant and wound healing evaluation of the extracts and spectral analysis for isolation and characterization of active compounds.
The research presented in this thesis focused on antimicrobial, antioxidant and wound healing action of such resource, as plants are recognized to be fairly resistant to most microbes and are traditionally used for ailments like wounds, burns etc. Therefore, specific focus was given on antimicrobial activity, antioxidant and wound healing activity.

7.1.1 Chapter 1 comprises of common introduction outlining antimicrobials used in human medication, agriculture, food and household goods in addition to clinical antibiotics used all over the planet. The over usage of antibiotics is root of the increase in microbial resistance, for that reason new antibiotics are desirable. Therapeutic plants are measured as an interesting basis for such original antimicrobial compounds.

7.1.2 Chapter 2 is an assessment of nearly all general modes of activity of antimicrobial agents. A brief description of Ipomoea family. The general information related to ipomoea species and all the data of the plant material used in the present investigation.

7.1.3 Chapter 3 shows the aim and objective of the present study.

7.1.4 In Chapter 4 we account antimicrobial action of the extracts which comprises of

7.1.4.1 Phytochemical screening
Phytochemical screening illustrated that the extracts of the plant is augmented in phytoconstituent contnt. The benzene extract of Ipomoea reniformis illustrated the existence of mucilages. The chloroform extract of Ipomoea reniformis illustrated the existence of reducing sugars, gum, steroids, glycosides, oxalic acids, tannins, flavonoids and phenolic compounds. The Ipomoea reniformis ethanolic extract illustrated the presence of alkaloids, oxalic acid, tartaric acid, malic acid, glycosides, tannin, flavonoids and phenolic compounds. The Ipomoea reniformis ethyl acetate extract illustrated the existence of phytoconstititution comparable to that of ethanolic extract except mucilage are additionally present in it. From the preliminary phytochemical investigation it was obvious that Ipomoea reniformis aqueous extract illustrated the existence of glycosides.

7.1.4.2 Antimicrobial Activity
The antimicrobial activity was executed for the entire crude extracts and various fractions of ethyl acetate extract of Ipomoea reniformis, the antimicrobial screening was performed for antibacterial (gram negative and gram positive microorganism) and antifungal action.

7.1.4.3 Antibacterial activity
The antibacterial activity was carried out by the agar well diffusion test using gram negative, gram positive bacteria and fungal strain. All extracts except chloroform failed to
inhibit growth of *A. niger*. Ethyl acetate extract inhibited all tested gram-negative, gram-positive bacteria with zone of inhibition 8.13mm against *E. coli*, 6.53mm against *P. aeruginosa*, 9.12mm against *S. aureus*, and 9.15mm against *B. Subtilis* but showed no effect on fungi *A. niger*. Ethanol extract also inhibited both gram-negative and gram-positive bacteria with zone of inhibition against *E. coli* 7.89mm, against *P. aeruginosa* 6.00mm, against *S. aureus* 7.97mm, and 7.58mm against *B. Subtilis* except fungi *A. niger*. Only chloroform extract showed activity against fungi with zone of inhibition 9.15mm. MIC values of all the extracts used for the study against different microorganisms. Ethyl acetate extract marked the lowest MIC values viz. 0.10 for *S. aureus*, 0.20 for *E. coli*, 0.39 for *P. Aeruginosa* and 0.10 for *B. subtilis*, after ethyl acetate the second most active extract was the ethanolic extract.

### 7.1.4.4 Chromatographic separation of potent crude extracts of *Ipomoea reniformis*

The most effective extract from the antimicrobial action was chosen for the column chromatographic division. The ethyl acetate extract of *I. reniformis* was processed further for the column chromatographic separation using various solvents in rising polarity (Benzene, chloroform, ethyl acetate, ethanol & aqueous) to attain the benzene, chloroform, ethyl acetate, ethanolic fraction and aqueous fractions. The dried ethyl acetate extract of plant materials was processed to column chromatography. The eluates were collected in grouped based on their thin layer chromatography (TLC) profile to yield 05 fractions, IPR-1 to IPR-5 and the antimicrobial and MIC study was carried out. From the results obtained it was clear that all fraction of ethyl acetate extract of *I. reniformis* posses antimicrobial activity, maximum activity was shown by ethyl acetate fraction with maximum zone of inhibition against *E.Coli* 9.57mm followed by, against *P. aeruginosa* with zone of inhibition of 8.40mm, and 11.02mm against *S. aureus* and 11.27mm was the zone of inhibition recorded for *B. Subtilis*. The second highest activity was shown by ethanolic fraction with highest zone of inhibition against *E.Coli* 6.31mm, against *P. aeruginosa* with zone of inhibition of 6.58mm, and 6.97mm against *S. aureus* and 6.41mm was the zone of inhibition recorded for *B. Subtilis* also it showed activity against *A. niger* with a zone of inhibition of 8.21 mm. The third most active fraction was chloroform fraction with highest zone of inhibition against *E.Coli* 6.04mm, against *P. aeruginosa* with zone of inhibition of 5.21mm, and 5.63mm against *S. aureus* and 6.14mm was the zone of inhibition recorded for *B. Subtilis* also it showed activity against *A. niger* with a zone of inhibition of 8.03mm.
The MIC values of all the fractions used for the study against different microorganisms. Ethyl acetate extract marked the lowest MIC values viz. 0.02 for *S. aureus*, 0.05 for *E. coli*, 0.20 for *P. aeruginosa*, 0.02 for *B. subtilis* and 12.50 for *A. niger*.

After data analysis it was evident that the most potent fraction was ethyl acetate fraction. Hence the ethyl acetate fraction was further sub-fractioned and cleansed by organic solvents to achieve the compound 1.

### 7.1.6 In Chapter 5 we report antioxidant and wound healing activities for the extracts

#### 7.1.6.1 Phenolic and flavonoid contents

The total phenolic content of all the extract, calculated from the calibration curve \((R^2 = 0.28)\), was 28.12 ± 1.08 gallic acid equivalents/g which was highest for ethanolic extract, 21.40 ± 0.32 gallic acid equivalents/g which was noted for chloroform extract followed by 16.84 ± 0.19 for aqueous and 12.58 ± 0.26 gallic acid equivalents/g for ethyl acetate and least was noted for benzene extract. And the total flavonoid content \((R^2 = 0.099)\) was 59.29 ± 1.87 rutin equivalents/g for ethanolic extract followed by 52.32 ± 0.69 rutin equivalents/g for chloroform and 29.15 ± 0.43, 24.54 ± 1.87, 6.64 ± 0.12 rutin equivalents/g respectively for Ethyl Acetate, Aqueous and Benzene extracts.

#### 7.1.6.2 Antioxidant activity

Antioxidant activity of crude extract of *I. reniformis* was studied using DPPH, Superoxide radical scavenging and α-Amylase inhibitory activity. All the extract illustrated the antioxidant action as compared with standard ascorbic acid and acarbose. The ethanolic extract of *I. reniformis* exhibited potent antioxidant activity for DPPH, Superoxide radical scavenging and Alpha-Amylase Inhibitory Activity method. Rest of the extracts illustrated a moderate activity as compared to the respective standard compound. The quantity of DPPH reduced could be measured by calculating the decrease in absorbance at 517 nm. The IC\(_{50}\) value was found to be 9.87±0.54 μg/ml\(^{-1}\), 23.65±1.98 μg/ml\(^{-1}\), 36.41±1.54, 42.21±1.31 μg/ml\(^{-1}\) and 58.26±0. μg/ml\(^{-1}\) for ethanolic, Ethyl Acetate, Aqueous, Chloroform and Benzene extracts while the IC\(_{50}\) value of Vitamin C was 4.12±0.21μg/ml significantly reduced DPPH radical by bleaching it. Superoxide radical scavenging method, the ethanolic extract again showed highest IC\(_{50}\) activity 96.66 μg/mL\(^{-1}\) among all the samples used followed by benzene extract with a value of 88.01 μg/mL\(^{-1}\). In the α-amylase inhibitory activity method, all the extracts exhibited potent antioxidant activity but
ethanolic extract again was most potent among all 102.32 µg/mL -1 followed by benzene extract with 135.72 µg/mL -1.

7.1.7 GC-MS Analysis of ethanolic extract
As from the result of wound healing and antioxidant activity it was evident that ethanolic extract posses active compound for the aforesaid activity. So the ethanolic extract was subjected to GCMS analysis. GCMS chromatogram study of the ethanolic extract of *I. reniformis* illustrated various peaks demonstrating the existence of seventeen phytochemical ingredients. While comparing the mass spectrum of the constituents with that of the NIST library, all the 17 phytocompounds were differentiated and recognized.

7.1.6.3 Wound healing activity

7.1.6.4 Excision wounds
The wound healing activity was done with all the extracts and fractions of potent extract. The animals treated with ethyl acetate extract of *I. reniformis* revealed the healing of wound completely in 16 days as compared with other extracts of *I. reniformis*. The epithelization period was found to be 16.43 ± 0.89** of ethyl acetate of *I. reniformis* relatively which was comparable to framycetin treated group (13.7 ± 1.52** days).

The animals treated with ethyl acetate fraction of *I. reniformis* revealed the healing of wound completely in 14<sup>th</sup> days as compared with other fraction of *I. reniformis*. The epithelization period was found to be 13.8±0.09** of ethyl acetate fraction of *I. reniformis* relatively which was comparable to framycetin treated group (13.7 ± 1.52** days).

7.1.6.5 Incision Wound model
The outcome of the incision wound healing study discovered that the breaking strength was found to be elevated in ethyl acetate extract treated group of *I. reniformis* 479.85 ± 12.92** g and it was equally potent to standard drug framycetin group 494.42 ± 15.82 g. All the test drugs in incision wound model showed to have major wound healing action. The best activity was noted for ethyl acetate extract with 479.85 ± 12.92 g reading followed by ethanolic extract with 377.97 ± 17.92 followed by Chloroform extract 347.57 ± 14.03 g and the least activity was shown by Aqueous extract treated group with a reading of 332.41 ± 13.21 g.
The incision wound healing study with fractions discovered that the breaking strength was found to be elevated in ethyl acetate fraction treated group of *I. reniformis* 492.59 ± 11.32 g and it was equally potent to standard drug framycetin group 494.42 ± 15.82 g.

7.1.6.6 Wound healing activity in infected wound
The ethyl acetate extract showed best activity in normal wound study so the ethyl acetate extract was tested against infected model by *S. aureus* and *P. aeruginosa*. The Wound healing action of the ethyl acetate extract of the *I. reniformis* (excision wound model) inoculated by *S. aureus* showed epithelialization period of 19.41 ± 1.08 days as compared to standard (18.21 ± 1.32) it showed a marked result. The Wound healing action of the ethyl acetate of the *I. reniformis* (excision wound model) inoculated by *P. aeruginosa* showed epithelialization period of 20.47 ± 0.69 days which is comparable to standard 18.93 ± 1.87 days.

7.1.7 In Chapter 6 we report; Isolation, Characterization and *In silico* study of the isolated compounds
Since the test report embodied in the thesis evidence that ethyl acetate extract and ethyl acetate fraction of ethyl acetate extract of *I. reniformis* shows comparatively better activity than other fractions, hence it enforced us to isolate compound present in the ethyl acetate fraction.
Ethyl acetate fraction of *I. reniformis* is done by column chromatographic techniques using different non-polar to polar solvents in different proportions and finally examined using TLC plate to get a single spot. The single spot containing solvent extract was eluted and dried to get the isolate product. The so obtained isolate product was analyzed by IR, UV, 1H NMR and MASS spectroscopic techniques, subsequently the spectral data were interpreted by referring previously reported information of similar spectral data and the isolate product is characterized as Scopoletin. The IUPAC name of the proposed compound is 7-Hydroxy-6-methoxy-2H-chromen-2-one.
The 3D structure of the scopoletin isolated from *I. reniformis* was docked with the *E. coli* FtsZ (Sridevi et al. 2014) and COX receptor found a good interaction by establishment of bonds between different site of the receptor and the isolate molecule (Chopade et al. 2015). The *in silico* docking interactions prove that the scopoletin can inhibit the receptors.
7.2 Conclusion
From the current study it is obvious that the ethyl acetate crude extract and ethyl acetate fraction acquired from column chromatographic separation of ethyl acetate crude extract of the plant *I. reniformis* showed best wound healing activity and exhibited antimicrobial action equally for bacterial and fungal species of microbes. The ethanolic extract showed best antioxidant activity and GCMS study revealed presence of 17 active moieties in it. The ethyl acetate crude extract and ethyl acetate fraction showed best wound healing activity (both in normal and infected wound model). The compound 1 isolated from the *I. reniformis* was scopoletin. Therefore, it can be suggested that the folkloric claims about the plant species are true and an additional exhaustive target based study should be done on these effective plant extracts which may direct to a novel chemical unit for eliminating the microbial associated sickness and additional pharmacological action for helping the human society, additional broad pharmacological examinations are desirable to explain the correct mechanism of activity of the plant material.