Original Research Article **Microbiology**

International Journal of Pharma and Bio Sciences

ISSN 0975-6299

STUDIES ON PHYTOCHEMICAL AND ANTIMICROBIAL ACTIVITY IN PISONIA GRANDIS R. Br.

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ABSTRACT

In the present study, phytochemical screening and antimicrobial activity of *Pisonia grandis R.Br.* have been studied. The *P. grandis* crude extracts were prepared by using different solvents (ethanol, methanol, acetone, chloroform and ethyl acetate).The phytochemical screening shows the presence of secondary metabolites such as alkaloids, phenols, sterol, flavonoids, diterpenes, cardiac glycosides, coumarins, tannins, steroids, terpenoids and saponin. Alkaloids, glycosides and terpenoids were absent in acetone, chloroform and ethyl acetate extract. Saponin was extracted in all solvent except Methanol. All phytoconstituents are present in ethanol extract. Antimicrobial activity was done against eight pathogenic bacteria [two are Gram positive (*Bacillus subtilis and Staphylococcus aureus)* and six are Gram negative (*E. coli, Proteus vulgaris, Pseudomonas aeruginosa* and *Lactococcus lactis, Micrococcus and Serratia marcescens)*].The antimicrobial activity was determined by Kirby–Bauer test and zone of inhibition was measured in cm. In all extract T_1 shows high activity than T_2. Among all the extract, ethanol showed good antimicrobial activity against all tested organisms next followed by methanol extract.

KEY WORDS: ***Pisonia grandis, antimicrobial activity, pathogens, phytochemicals, bacteria.***

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Received on: 27-06-2018
Revised and Accepted on: 29-09-2018
DOI: http://dx.doi.org/10.22376/ijpbs.2018.9.4.b213-220

INTRODUCTION

Bacterial species are responsible for mortality in human population because their infection causes diseases like food borne gastroenteritis, secondary infections, mastitis, upper respiratory complications, urinary tract infection, diarrhea, septicemia (*E.coli, Proteus vulgaris)*, skin infection, acute toxemia and pneumonia (*Staphylococcus aureus*). Treatment of infectious diseases is a big challenge due to multi-drug resistance, as pathogens gets resistance to existing antibiotics. It is therefore highly desirable to explore plants for new alternative antimicrobial agents to treat infectious diseases[1] Organisms that cause infections are called as pathogens In table I shows the microorganisms and its infections which are used in our present study.

Table 1
Microorganism and its infections

Microorganisms	Infection
Bacillus subtilis [Gram positive]	Gastrointestinal tract, skin infections
Staphylococcus aureus [Gram positive]	Endocarditits, Ostecomyelitis, skin infection
Micrococcus [Gram Negative]	Leukemia, Septic shock and Septic arthritis
Serratia marcescens [Gram Negative]	Conjuctivitis, keratitis, Pneumonia
E.coli [Gram Negative]	Gastro enteritis, septicemia, Mastitis
Proteus vulgaris [Gram Negative]	Urinary tract infection, wound infection
Pseudomonas aeruginosa [Gram Negative]	Opthalamia, Conjectiva, Noscomial infection
Lactococcus lactis [Gram Negative]	Endocarditic

Bioactive compounds are normally accumulated as secondary metabolites in all plant cells but their concentration varies according to the plant parts, season climate and particular growth phase.[2] Leaf is one of the highest accumulated plant part of such compounds and people prefer it for therapeutic purposes some of the active compounds inhibit the growth of disease causing microbes either single or in combination.[3] One such important medicinal herb is *Pisonia grandis R.Br.* It is commonly known as "Leechai Kottai Keerai" in Tamil and Lettuce tree in English. According to traditional use, the different parts of the plants of *P. grandis* are used as diuretics and purgative. The anti-inflammatory, antifungal and wound healing and diabetic activities of *P. grandis* were reported earlier.[4-6] Hence in the present study, an attempt has been made for the qualitative and quantitative analysis of its secondary metabolites and also evaluate the antimicrobial activity of *P. grandis R.Br* leaves.

MATERIALS AND METHODS

The present research aimed at the development of antimicrobial agents from natural sources. The plant material chosen based on the properties were collected from the local area in and around Madurai and Virudhunagar District. The leaves were washed in running water and the leaves were dried in shade in an airy place and then stored in polythene bags at room temperature. At the time of experiment, the dried leaves were powdered by using an electric blender and the powder was used for extraction procedure.

Extraction procedure[7]

About 20g of dried leaf powder was extracted using 200ml of solvent (ethanol, methanol, acetone, chloroform and ethyl acetate) at 37°C using cold apparatus for 8 hours. The extract was concentrated under vacuum.

Qualitative analysis of phytochemicals

Different solvent leaves extracts were evaluated for preliminary screening of secondary phytochemicals such as, alkaloids, phenols, sterol, flavonoids, diterpenes, cardiac glycosides, coumarins, tannins, steriods, terpenoids and saponin.[8]

Quantitative analysis of phytochemicals

The amount of carbohydrate[9], protein[10,11], phenol[12], tannins[13],alkaloids[14], flavanoids[14], anthocyanins[15], ascorbic acid [16] present in the different solvent extract was estimated.

Microorganisms

Eight bacterial cultures namely *Bacillus subtilis, Serratia marcescens, Micrococcus, Staphylococcus aureus, E.coli, Proteus vulgaris, Pseudomonas aeruginosa* and *Lactococcus lactis* were chosen for this study. All the cultures were procured from VHNSNC, Virudhunagar, Tamilnadu, India. All strains were cultivated and maintained in Luria–Bertani (LB) medium (pH 7.0) at 37°C. Overnight cultures of the above mentioned strains were used for this study.

Antimicrobial assay

Antimicrobial bioassay was carried out by Kirby–Bauer method. Muller Hinton agar medium (MHA) (25 ml) was distributed in each 100ml petriplates under sterilized condition. Using a sterile cotton swab, the test pathogens were uniformly spread over the surface of the MHA plates. After spreading, three wells were made by scooping out the medium with a sterile cork borer (0.5cm).100 µl of test solutions of various concentrations (200µg and 100µg) were placed in two wells and considered as T_1 and T_2 respectively and the third well was filled with the solvent alone which acted as solvent control(C) fourth well Ampicillin (100µl) was added as standard. The plates were allowed to diffuse at room temperature for two hours and then incubated in the upright position at 37°c for 18 hours. Each experiment was carried out and the diameter of inhibition zone was measured in centimeter and recorded.

STATISTICAL ANALYSIS

All experiments were independently repeated three times. The data obtained were analyzed using GraphPad Prism 5.03 (GraphPad Software, Inc.; USA) software.

RESULTS AND DISCUSSION

The results of the preliminary phytochemical screening of the ethanol extract revealed the presence of all the phytoconstituents such as alkaloids, sterols, protein, carbohydrates, tannin, quinine, flavanoids, phenol and coumarin (Table 2). Our results coincides with Elumalai *et al.,* 2012.[17] Alkaloids, glycosides and terpenoids were absent in acetone, chloroform and ethyl acetate extract. Saponin was extracted in all solvent except methanol. The analysis of the plant extracts revealed the presence of phytochemicals which are known to exhibit medical and biological activities. For example, tannins are polyphenolic compounds that bind to proline rich proteins of pathogens and that interferes with protein synthesis.[18-20]

Table 2
Phytochemical screening of Pisonia grandis R.Br. leaves

S.No.	Phytochemical constituents	Ethanol	Acetone	Chloroform	Ethylacetate	Methanol
1	Protein	+	+	+	+	+
2	Carbohydrate	+	+	+	+	+
3	Sterols	+	+	+	+	+
4	Alkaloids	+	-	-	-	+
5	Flavanoids	+	+	+	+	+
6	Quinone	+	+	+	+	+
7	Fatty acid	+	+	+	+	+
8	Tannin	+	+	+	+	+
9	Terpenoids	+	-	-	-	+
10	Phenol	+	+	+	+	+
11	Saponins	+	+	+	+	-
12	Glycosides	+	-	-	-	-
13	Coumarin	+	+	+	+	+
14	Xanthoproteic acid	+	+	+	+	+

+ =Presence ; - = absent

Flavanoids are hydroxylated polyphenolic compounds known to be produced by plants in response to microbial infections to which this aspect has been extensively studied and found to have antimicrobial activity against an array of microorganism *in vitro*.[21] Their ability has been attributed to their ability to form complexes with extracellular and soluble proteins and bacterial cell walls. Terpenoids were mainly used for their aromatic qualities. They have also been found to be potential agents against inhibiting bacteria.[22]

Table 3
Quantitative analysis of secondary metabolites in different solvent extracts of P. grandis

S.No.	Compound	Ethanol (mg/g)	Methanol (mg/g)	Acetone (mg/g)	Chloroform (mg/g)	Ethylacetate (mg/g)
1	Carbohydrates	3.21±0.05	1.69± 0.21	1.18± 0.09	0.76± 0.14	0.67± 0.23
2	Protein	9.8± 0.55	7.30± 0.16	5.10± 0.57	9.20± 0.18	4.8± 0.38
3	Phenol	1.85± 0.32	1.70± 0.18	1.10± 0.48	0.35± 0.61	1.45± 0.12
4	Tannin	11.9± 0.12	10.8± 0.23	5.8± 0.34	2.70± 0.37	8.5± 0.66
5	Flavanoids	3.8± 0.07	3.2± 0.41	1.0± 0.62	1.90± 0.08	1.3± 0.11
6	Alkaloids	7.8± 0.38	7.0± 0.52	6.0± 0.49	4.60± 0.46	5.8± 0.33

The data are expressed in mean ± SEM; n=3.

Table 4
Antimicrobial activity of different extracts of P. grandis leaves

Bacterial. Strains	Ethanol (cm)			Methanol (cm)			Acetone (cm)			Chloroform (cm)			Ethylacetate (cm)		
	T_1	T_2	A	T_1	T_2	A	T_1	T_2	A	T_1	T_2	A	T_1	T_2	A
Bacillus	0.7±0. 20	0.6±0.10	-	0.5±0.12	0.4±0.08	-	0.6±0.05	0.5±0.08	-	0.3±0.11	0.2±0.10	1±0.28	-	-	-
S. aureus	0.9±0.58	0.5±0.15	0.3±0.04	0.6±0.16	0.5±0.13	-	0.7±0.19	0.6±0.03	-	0.4±0.15	0.3±0.08	0.2±0.06	0.7±0.14	0.6±0.21	0.5±0.14
Lactococcus	0.7±0.14	0.6±0.20	-	0.6±0.07	0.5±0.09	0.2±0.05	0.4±0.21	0.3±0.10	-	0.5±0.23	0.4±0.11	-	0.4±0.09	0.3±0.12	-
Proteus	0.9±0.18	0.6±0.12	-	0.5±0.18	0.4±0.07	0.1±0.02	0.5±0.15	0.4±0.13	0.1±0.05	0.3±0.12	0.2±0.08	0.2±0.05	0.5±0.13	0.4±0.10	-
P. aeruginosa	0.5±0.08	0.3±.01	0.1±0. 02	0.4±0.21	0.3±0.11	0.2±0.02	0.3±0.09	0.2±0.08	-	0.5±0.03	0.4±0.13	-	0.5±0.16	0.3±0.08	-
Micrococcus	0.4±0.21	0.3±0.06	0.4±0.03	0.5±0.09	0.4±0.23	0.2±0.04	0.5±0.07	0.3±0.01	0.1±0.04	0.4±0.16	0.3±0.02	0.9±0.15	0.4±0.09	0.3±0.05	0.5±0.06
S. marcescens	0.4±0.12	0.3±0.16	0.5±0.08	0.3±0.14	0.2±0.10	-	0.4±0.06	0.3±0.07	0.4±0.12	0.3±0.08	0.1±0.05	0.4±0.11	0.5±0.18	0.4±0.12	0.5±0.01
E. coli	0.5±0.16	0.4±0.08	-	0.3±0.05	0.2±0.07	0.2±0.03	0.2±0.02	0.1±0.01	-	0.3±0.04	0.2±0.05	-	0.3±0.09	0.1±0.10	-

The data are expressed in mean ± SEM; n=3.

Table 3 represents the quantitative analysis of the *P. grandis* leaves. It shows the ethanolic extract was rich in carbohydrate content. High amount of protein was present in ethanol extract. Phenol content was very low in chloroform extract than the other extract. Tannin was present in higher amount in ethanol extract. In acetone extract, low amount of flavanoids content was estimated. This result coincides with Ayitey and addae, 1977.[23] Flavonoids content of leaves of *Gymnema sylvestre* was found to be 4.7 to 0.127 in ethanol extract. Flavonoids content was present in low amount of *A. indica* found to be 0.62, 0.10 and 0.52 to 0.20. Alkaloids were rich in ethanol extract than the other extracts. This result coincides with the alkaloid content of *Carica Papaya* which was found to be 1.22 to 0.060 for ethanol extract.[23] Carbohydrates performs numerous roles in living organisms. Polysaccharides serve for the storage of energy (e.g., starch and glycogen), and as structural components (eg. Cellulose in plants and chitin in arthropods).Phenolics are the most widely spread secondary metabolites in plant kingdom. The interests of phenolics are increasing in the food industry because they retard oxidative degradation of lipids and improve the quality and nutritional value of food. The presence of phenolic compounds in the leaves of *P. grandis* showed that the leaf may have antimicrobial potential. This explains its use in treating diarrhea, typhoid fever and some other intestinal problem. This is because phenols and phenolic compounds have been extensively used in disinfections. The biological functions of Flavonoids include protection against allergies inflammations, platelets aggregation by microbes, ulcer and tumors.[24] Flavonoids are present in the common and widely distributed group of plant phenolics. They are free radical scavengers and super antioxidants and potent which prevent oxidative cell damage and have strong anticancer activity.[25] This may be the reason behind the use of leaf extracts of *P. grandis in* herbal medicines. Alkaloids are the most efficient therapeutically significant plant substance.[26] Alkaloids are also central nervous system stimulants. They have anthelmintic properties and serve as aphrodisiacs in the treatment of erectile dysfunction.[27] The tannin may be partly responsible for the bitter astringent property associated with the leaf extracts of *P.grandis*. Table 4 represents the antibacterial activity of the *P. grandis* leaves extract. Ethanol extract showed high antibacterial activity against *Bacillus, Staphylococcus, Lactococcus, Proteus* and *E. coli.* The results were concordant with the observations of previous study by researchers.[28] However, the result of ethyl acetate extract was comparative to those of other authors *Jeenu joseph et al., 2011*[29] who had reported reduced activity of extract on microbes. *E.coli* suggesting that Gram negative organisms may be better equipped naturally to prevent the action of water extract.

Antimicrobial activity of ethanolic extracts

The antimicrobial activity of ethanolic extracts of *P. grandis* on eight different human pathogenic organisms were studied using Agar well diffusion assay using two different concentrations of extracts. T_1 (200µg) concentration of extract have showed maximum zone of inhibition (0.9cm) against *Staphylococcus* which is near to the observations of *Rashmi Chandra et al., 2011*[30] *where in they* reported that the ethanol extract of *Ocimum sanctum* on *staphylococcus* (13mm) followed by *Micrococcus* (0.6cm). The collective analysis of antimicrobial activity of ethanolic extract of *Pisonia grandis R.Br* have better impact ranged from 0.2 to 0.9cm on all the eight species of pathogenic bacteria Whereas, in case of T_2 concentration (100µg), the ethanolic extract of *Pisonia grandis R.Br* showed the maximum zone of inhibition against *Bacillus* (0.7cm) followed by *Serratia* (0.3cm), *Bacillus* (0.4cm), *Micrococcus* (0.4cm), *E. coli* (0.4cm), *Pseudomonas* (0.3cm), *Proteus* (0.6cm) *Staphylococcus* (0.5cm) and *E.coli*(0.4cm) with the extract of *Pisonia grandis R.Br.* The ethanol extract of *P. grandis shows* high as well as somewhat equal zone of inhibition compared to all the medicinal plants discussed above. This shows that *P. grandis* is one of the very good medicinal plants showing better antibacterial activity.

Antimicrobial activity of methanolic extract

The antimicrobial activity of methanolic extracts of *P. grandis* on eight different human pathogenic organisms using two different concentrations of extract. T_1 (200µg) concentration of extract have showed maximum zone of inhibition (0.6cm) against *Staphylococcus* and *Lactococcus* followed by *Micrococcus* (0.5cm) which is near to the activity observed by *Umamaheswari et al.,2008*[28] was reported that the Methanol extract of *B. spectabilis* on *Micrococcus* – 7mm, *Bacillus* (0.5cm), *Serratia* (0.3cm), *Pseudomonas* (0.4cm) *Proteus* (0.5cm) and *E. coli* (0.3cm). The collective analysis of antimicrobial activity of methanolic extract of *P. grandis* have better impact ranged from 0.2 to 0.6cm on all the eight species of pathogenic bacteria Whereas, in case of T_2 concentration (100µg), the Methanolic extract of *P. grandis* showed the maximum zone of inhibition against *Staphylococcus* and *Lactococcus* (0.5cm) followed by *Serratia* (0.2cm), *Micrococcus* (0.4cm), *Bacillus* (0.4cm), *Pseudomonas* (0.3cm), *Proteus* (0.4cm) and *E. coli* (0.2cm) with the extract. The methanol extract of *P. grandis R.Br* shows high as well as somewhat equal zone of inhibition compared to all the medicinal plants discussed above. This shows that *P. grandis R.Br* is having good antibacterial activity.

Antimicrobial activity of Chloroform extract

The antimicrobial activity of Chloroform extracts of *Pisonia grandis R.Br* on T_1 concentration showed maximum zone of inhibition against *Lactococcus* and *Pseudomonas* 0.5cm followed by *Staphylococcus* (0.4cm), *Micrococcus* (0.5cm) which is close to the *Umamaheswari et al.,2008*[28] reports of the Chloroform extract of *B. spectabilis* on *Pseudomonas* *(* 8mm). The inhibition Zone of chloroform extract shows *Serratia* (0.3cm), *Bacillus* (0.3cm), *Proteus (*0.3cm) and *E. coli* (0.3cm). Similar result was reported in the Chloroform extract of *Cleodenrum paniculatum Linn.*[29] The collective analysis of antimicrobial activity of chloroform extract of *P. grandis* have better impact ranged from 0.2 to 0.5cm on all the eight species of pathogenic bacteria Whereas, in case of T_2 concentration (100µg), the chloroform extract of *P. grandis* showed the maximum zone of inhibition against *Lactococcus* and *Pseudomonas* (0.4cm) followed by *Serratia* (0.1cm), *Micrococcus* (0.3cm), *Bacillus* (0.2cm) *Staphylococcus* (0.3cm), *Proteus* (0.2cm) and *E. coli* (0.2cm) with the extract of *P. grandis.* The chloroform extract of *P. grandis* shows

high as well as somewhat equal zone of inhibition compared to all the medicinal plants discussed above. This shows that *Pisonia grandis R.Br* can be used for treating many human diseases caused by human pathogenic microbes.

Antimicrobial activity of Acetone extract

The antimicrobial activity of acetone extracts of *P. grandis* on T_1 concentration showed maximum zone of inhibition against *Bacillus* and *Staphylococcus* (0.4cm) followed by *Micrococcus* (0.3cm), *Serratia* (0.3cm), *Proteus* (0.5cm), *E. coli* (0.2cm) which is near to the *Pavithravani et al.,2010*[31] was reported that the acetone extract of *Piper nigrum Linn* on *E.coli* – 5mm, *Lactococcus* (0.4cm) and *Pseudomonas* (0.3cm). The collective analysis of antimicrobial activity of acetone extract of *P. grandis* have better impact ranged from 0.2 to 0.5cm on all the eight species of pathogenic bacteria Whereas, in case of T_2 concentration (100µg), the Acetone extract of *P. grandis* showed the maximum zone of inhibition against *Bacillus* and *Staphylococcus* (0.4cm) followed by *Lactococcus* (0.3cm) *Serratia* (0.3cm), *Micrococcus* (0.3cm), *Bacillus* (0.2cm) *Pseudomonas* (0.2cm), *Proteus* (0.4cm) and *E. coli*(0.1cm) with the extract of *P. grandis.* The Acetone extract of *P. grandis* shows high as well as somewhat equal zone of inhibition compared to all the medicinal plants discussed above. This shows that *P. grandis* exhibits moderate antibacterial activity.

Antimicrobial activity of Ethyl acetate extract

The antimicrobial activity of Ethyl acetate extracts of *P. grandis* on eight different human pathogenic organisms using Agar well diffusion on two different concentration of extract was studied. T_1 (200µg) concentration of extract have showed maximum zone of inhibition (0.7cm) against *Staphylococcus* followed by *Serratia* (0.5cm), *E.coli* (0.3cm), *Micrococcus* (0.4cm), *Bacillus* no Zone of inhibition, *Proteus* (0.5cm), *Pseudomonas* (0.5cm) which is closer to the *Umamaheswari et al.,2008*[28] reported that the ethyl acetate extract of *Ocimum tenuiflorum*on *Pseudomonas* and *Lactococcus* (0.4cm). The collective analysis of antimicrobial activity of aqueous extract of *P. grandis* have better impact range from 0.2 to 0.7cm on all the eight species of pathogenic bacteria Whereas, in case of T_2 concentration (100µg), the Ethyl acetate extract of *P. grandis* showed the maximum zone of inhibition against *Staphylococcus* (0.6cm) followed *Bacillus* no zone of inhibition *Serratia* (0.4cm), *Micrococcus* (0.3cm), *Bacillus* (0.2cm) *Pseudomonas* (0.3cm), *Proteus* (0.4cm) and *E. coli* (0.2cm) with the extract of *P. grandis.* The Acetone extract of *P. grandis* shows high as well as somewhat equal zone of inhibition compared to all the medicinal plants discussed above. This shows that *P. grandis* moderate activity of antibacterial activity. The Ethanol extract had a significant effect on all the pathogen and it was high activity against *Bacillus subtilis, Staphylococcus species, Serratia and E. coli* in ethanol extract of *P. grandis R.Br.* Methanol and Acetone extract of the leaves of *P. grandis* exhibit moderate activity against all the tested bacterial strains. Chloroform and Ethyl acetate extract of the leaves revealed the t low activity against all the tested bacterial strains. Medicinal plants are a source for novel drugs. Medicines derived from plants have made great contribution to human health. Traditional healers make use of water primarily and secondarily as a solvent but ethanolic extract of this plant were contain much better and powerful. This may be due to the better solubility of active components organic solvent.[32] Several works have been documents on the pharmacological screening of the plant extract which have been exploited as the source of innumerable therapeutic agents.[33-39]. The ethanobotanical approach assumes that the population use of plants that can offer strong includes to the biological activity of plant. The high percentage of positive result found in this study shows that this approach is also promising for antimicrobial activity. The result of the present study reveals fact that the organic solvent extract (Ethanol) exhibited greater antimicrobial activity. Bacterial pathogen gram positive bacterial strains were found to be more susceptible than gram negative bacterial strains. The antibacterial activity may be due to the presence of several metabolites from herb species including tannin sterols have associated with antibacterial activity.[34] Several reports have been shown that bioactive compound present in the plant extract have inhibitory effect on pathogen strains.[40-43]

CONCLUSION

In the present study, phytochemical analysis (qualitative and quantitative) and antimicrobial property of the *P. grandis* were investigated. The ethanol extract contains more phytoconstituents and also showed good growth inhibitory effect against all tested organisms.

AUTHORS CONTRIBUTION STATEMENT

Salini.R and Sudharameshwari.K designed the experiments. Suganya.M performed the experiments. Sudharameshwari.K, Salini.R and Suganya.M analyzed the data and prepared the manuscript. All authors read and approved the final manuscript

ACKNOWLEDGEMENT

The authors are thankful to the Principal and the Management of V. V. Vanniaperumal College for Women, Virudhunagar, Tamil Nadu, India for providing necessary facilities to carry out this research work.

CONFLICT OF INTEREST

Conflict of interest declared none.

REFERENCES

1. Frey FM, Meyers R. Antibacterial activity of traditional medicinal plants used by Haudenosaunee peoples of New York State. BMC Complement Altern Med. 2010;10(1). DOI: 10.1186/1472-6882-10-64
2. MM. C. Plant products as antimicrobial agents. Clin Microbiol Rev. 1999;12(4):564–82. Available from: http://cmr.asm.org/content/12/4/564.full.pdf
3. DH,. AK. Antimicrobial Activity of Some Medicinal Plants Against Candida albicans. J Biol Sci. 2006;6(1):109–14. DOI: 10.3923/jbs.2006.109.114
4. Anbalagan, N., Rajinikanth, KN., Kishore Gnanasam, S., Thomas Leonard, J., Balakrishna, K., Ramachandran, S. and Sridhar, SK. Analgesic, Anti-inflammatory and Diuretic Activities of *Pisonia grandis*. J. Natural Product sci. 2002, 8(3):97- 99.
5. Radha R, Arokiyaraj S, Agastian P, Balaraju K, Mohan Kumar R B. Phytochemical analysis and anti-inflammatory activity of Pisonia grandis R.Br. J Biomed Pharmacol. 2008;1(1):127–30. Available from: http://biomedpharmajournal.org/vol1no1/phytochemical-analysis-and-anti-inflammatory-activity-of-pisonia-grandis-r-br/
6. Shubashini K. Sripathi and Poongothai G. Bioassay- guided fractionation and anti-fungal activity studies on Pisonia grandis.R.Br. Int J Pharmacogn Phytochem Res. 2013;5(1):1–3. Available from: http://impactfactor.org/PDF/IJPPR/5/IJPPR,Vol5,Issue1,Article1.pdf
7. JB Harborne. Phytochemical Methods. 2rd ed,. Chapman A and Hall, London: A Guide to Modern Techniques of Plant analysis; 1973. 49-188 p. Availble from: https://www.researchgate.net/profile/Houda_Kawas/post/How_to_dry_mushrooms_without_contamination/attachment/59d63aa879197b8077997c20/AS%3A406964409520134%401474039285395/download/%5BJ._B._Harborne_%28auth.%29%5D_Phytochemical_Methods_A.pdf
8. Kokate CK, Purohit AR GS. Pathway to Screen Phytochemical nature of Natural Drugs. Pharmacognosy,. 42nd ed. Nirali Prakashan Pune; 2008. 56-61 p.
9. Dubois m, gilles k, hamilton jk, rebers pa, smith f. A Colorimetric Method for the Determination of Sugars. Nature. 1951;168(4265):167. DOI: 10.1038/168167a0
10. Lowry OH, Rosebrough NJ, Farr AL RRJ. Protein measurement with Folin phenol reagent. J Biol Chem. 1951; 193(1):265–75.Available from: http://devbio.wustl.edu/InfoSource/ISPDFs/Lowry%201951.pdf
11. Mattoo RL. Influence of sodium chloride on the colorimetric determination of protein in plant tissues rich in phenols-phenolases. Indian j Biochem. 1970 Mar;7(1):82-3.
12. Malick CP SM. In: Plant Enzymology and Histo Enzymology. Kalyani Publishers, New Delhi; 1980. 286 p.
13. Burns RE. Method for Estimation of Tannin in Grain Sorghum1. Agron. 1971;63(3):511. DOI:10.2134/agronj1971.00021962006300030050x
14. Evans and Trease. Pharmacognasy, 14th ed. London: WB Saunders;1996; Available from: http://www.powells.com/book/trease-evans-pharmacognosy-14th-edition-9780702018992
15. Swain T, Hillis WE. The phenolic constituents ofPrunus domestica. I.—The quantitative analysis of phenolic constituents. J Sci Food Agric. 1959;10(1):63–8. DOI: 10.1002/jsfa.2740100110
16. Roe HJ, Kuether CA. Detection of ascorbic acid.J Biol Chem. 1943, 14(7): 399-407 Available from: http://www.jbc.org/content/147/2/399.full.pdf
17. Elumalai A, Chinna Eswaraiah, YetcharlaVenkatesh BS kumar and CN. Phytochemical and pharmacological profile Of Ixoracoccinea Linn. J Pharm Life Sci. 2012;3(3):1563-67. Available from: http://www.ijplsjournal.com/issues%20PDF%20files/march%202012/11.pdf
18. Sanches NR, Garcia Cortez DA, Schiavini MS, Nakamura CV, Dias Filho BP. An evaluation of antibacterial activities of Psidium guajava (L.). Brazilian Arch Biol Technol. 2005;48(3):429–36. DOI:10.1590/s1516-89132005000300014
19. Tsuchiya H, Sato M, Miyazaki T, Fujiwara S, Tanigaki S, Ohyama M, et al. Comparative study on the antibacterial activity of phytochemical flavanones against methicillin-resistant Staphylococcus aureus. J Ethnopharmacol. 1996;50(1):27–34. DOI:10.1016/0378-8741(96)85514-0
20. Ulubelen A. Cardioactive and antibacterial terpenoids from some Salvia species. Phytochemistry. 2003;64(2):395–9. DOI: http://dx.doi.org/10.1016/s0031-9422(03)00225-5
21. Cowan.M.M. Plant products as antimicrobial agents,. Clin Microbiol Rev. 1999;12(4):564–82. Availbale from: http://cmr.asm.org/content/12/4/564.full.pdf
22. Trease GE, Evans WC. Pharmacognosy. 1989; 11th edition Bailliere Tindall, London. 45-50. Available from:http://www.scirp.org/(S(351jmbntvnsjt1aadkposzje))/reference/ReferencesPapers.aspx?ReferenceID=1964412
23. Ayitey-Smith E A-MI. Phytochemical, nutritional and medical properties of some leafy vegetables consumed by Edo people of Nigeria. African J Biotechnol. 2008;7(14):2304–9. Available from: https://www.ajol.info/index.php/ajb/article/download/58988/47301
24. Me odo. Chemical Composition of Spondias. mornbin Plants. J Sustain Agri Env [Internet]. 2004;6(2):140–7. Available from: https://www.researchgate.net/publication/285751974_Chemical_composition_of_Spondias_mombin_Linn_plants_parts
25. Salah N, Miller NJ, Paganga G, Tijburg L, Bolwell GP, Riceevans C. Polyphenolic Flavanols as Scavengers of Aqueous Phase Radicals and as Chain-Breaking Antioxidants. Arch Biochem

Biophys. 1995;322(2):339–46. DOI:10.1006/abbi.1995.1473
26. Igboko DO. Phytochemical studies on Garcinia kola Heckel. MSc Thessis. University of Nigeria Nsukka. 1983;202.
27. Ostlund RE, Racette SB, Stenson WF. Inhibition of cholesterol absorption by phytosterol-replete wheat germ compared with phytosterol-depleted wheat germ. Am J Clin Nutr. 2003;77(6):1385–9. DOI: 10.1093/ajcn/77.6.1385
28. Umamaheswari A SAN. In vitro antibacterial activity of Bougainvillea spectabilis leaves extracts.; Adv Biol Res [Internet]. 2008;2(1–2):1–5. Available from: https://pdfs.semanticscholar.org/2de6/8ded7b36473f783327cb6410305959580a3d.pdf
29. Jeenu Joseph, Bindhu AR AN. Antimicrobial activity of Clerodendrum paniculatum linn. Leaves. IJRAP. 2011;2(3):1003–4. Available from: https://www.researchgate.net/publication/236681891_Antimicrobial_activity_of_Clerodendrum_paniculatum_Linn_leaves
30. Rashmi Chandra V, Dwivedi .Shivam K JA. Detectionof antimicrobial activity of Oscimum sanctum (Tulsi) & Trigonellafoenum graecum (Methi) against some selected bacterial &fungal strains. Res J Pharm Biol Chem Sci. 2011;2(4):809–13. Available from: https://www.researchgate.net/publication/269107198_Detection_of_Antimicrobial_Activity_of_Oscimum_sanctum_Tulsi_Trigonella_foenum_graecum_Methi_against_some_selected_bacterial_fungal_strains
31. PavithraVaniKarsha BO. Antibacterial of Black Pepper (Piper nigrum linn.)with special reference to its mode of action on bacteria". Indian J Nat Prod Resour. 2010;1(2):213–5. Available from: http://nopr.niscair.res.in/bitstream/123456789/9828/1/IJNPR 1%282%29 213-215.pdf
32. Kumar BSA, Lakshman K, TirupathiMS, Jayaveera KN, Narayanasamy VB, Sabemulla K. Nandeesh R. Free radical scavenging and antibacterial activities of Armycard power (An Ayurvedic formulation). European Bulltien of Drug Research. 2009.17:5-9. Available from: https://www.tfljournal.org/images/articles/20090211034141277_2.doc
33. Sudharameshwari K KK. Pharmacological activity of silver nanoparticles synthesized using Stemodia viscosa aqueous extract. IJPSR. 2018;9(3):1106–11. Avilable from: http://ijpsr.com/bft-article/pharmacological-activity-of-silver-nanoparticles-synthesized-using-stemodia-viscosa-aqueous-extract/?view=fulltext
34. Sakagami Y, Murata H, Nakanishi T, Inatomi Y, Watabe K, Iinuma M, et al. Inhibitory Effect of Plant Extracts on Production of Verotoxin by Enterohemorrhagic Escherichia coli O157 : H7. J Heal Sci. 2001;47(5):473–7. DOI: 10.1248/jhs.47.473
35. Herrera RM, Pérez M, Martín-Herrera DA, López-García R, Rabanal RM, Arias A. Antimicrobial Activity of Extracts from Plants Endemic to the Canary Islands. Phyther Res. 1996;10(4):364–6. DOI:10.1002/(sici)1099-1573(199606)10:4%3C364::aid-ptr846%3E3.3.co
36. Natarajan E, Senthilkumar S FXTK V. Antibacterial activities of leaf extracts of Alangium salviifolium. JTropl MedPlants. 2003;4(1):9–13.
37. Rameshwari K sudh., M A. Evaluation of antiulcerogenic activity of methanol extracts of brassica oleracea var. Capitata rubra on albino rat gastric ulceration. Asian J Pharm Clin Res. 2017;10(3):314. DOI:10.22159/ajpcr.2017.v10i3.16217
38. Ayshwarya S and. Anti-inflammatory and antipyretic activity of methanol extracts of Brassica oleracea var. Capitata rubra in wister rats. Int J Curr Res [Internet]. 2016;8(03):27299–302. Available from: http://www.journalcra.com/article/anti-inflammatory-and-antipyretic-activity-methanol-extracts-brassica-oleracea-var-capitata-
39. SudhaRameshwari K, Kaleeswari M TA. Wound healing and antibacterial activities of the chloroform extract of Glinus lotoides linn. in albino rats. Int J Pharm Bio Sci. 2013;4(4):722–8. Available from: http://www.ijpbs.net/cms/php/upload/2760_pdf.pdf
40. Leven M, Berghe D, Mertens F, Vlietinck A, Lammens E. Screening of Higher Plants for Biological Activities I. Antimicrobial Activity. Planta Med. 1979;36(08):311–21. DOI: 10.1055/s-0028-1097277
41. Ayshwarya M and SudhaRameshwari K. Antimicrobial activity of the plant extracts of Brassica oleracea var. Capitata rubra. J Int Acad Res Multidiscip. 2015;3(10):149–56. Available from: http://www.jiarm.com/NOV2015/paper25442.pdf
42. Thiruppathi S, Ramasubramanian V, Sivakumar T, Thirumalaiarasu V. Antimicrobial activity of Aloe vera (L.) Burm. f. against pathogenic microorganisms. J Biosci Res. 2010;1(4):251-8.
43. Karthigaiselvi K SK. Green synthesis of silver nanoparticles from aqueous extract of Stemodia viscosa and its Evaluation of antimicrobial activity. Eur J Pharm Med Res. 2016;3(12):417–21. Available from: http://www.ejpmr.com/admin/assets/article_issue/1480493317.pdf