



PHYTOCHEMICAL INVESTIGATION OF PLANTS LIMONIA ACIDISSIMA (L.) AND PERGULARIA DAEMIA (L.) FROM PRAKASAM DISTRICT, ANDHRA PRADESH, INDIA

P. Brahmam^{1*}, K. Sunita² and B. Hari Babu³

^{1,2}Department of Zoology and Aquaculture, Acharya Nagarjuna University, Nagarjuna Nagar 522510, Guntur, Andhra Pradesh, India.

³Department of Chemistry, Acharya Nagarjuna University, Nagarjuna Nagar 522510, Guntur, Andhra Pradesh, India.

***Corresponding Author: K. Sunita**

Department of Zoology and Aquaculture, Acharya Nagarjuna University, Nagarjuna Nagar 522510, Guntur, Andhra Pradesh, India.

Article Received on 18/03/2018

Article Revised on 08/04/2018

Article Accepted on 29/04/2018

ABSTRACT

Plants are sources of large amount of drugs to treat different ailments such as fever, emetics, cancer and bacterial infections etc. The present study reports the phytochemical analysis of leaf, stem bark and root extracts with chloroform, ethyl acetate and methanol solvents from *Limonia acidissima* (Groff) and hexane chloroform and methanol extracts of the *Pergularia daemia* (Forsk) plants. belonging to different families collected from Pedda Dornala, Prakasam district, Andhra Pradesh. The preliminary phytochemical screening was performed from these extracts for the presence of alkaloids, saponins, terpenoids and steroids, tannins, phenolic compounds, flavanoids, coumarins, quinones, resins, and glycosides.. The presence of alkaloids, saponins, terpenoids and steroids, tannins, anthocyanidin, phenolic compounds, flavanoids, coumarins, quinones, resins, and glycosides in all these extracts indicates that they could be used for the treatment of burns and wounds. Finally, the presence of high alkaloid flavonoids and terpenoids of the plant extracts suggest their antioxidant potential and justifies their therapeutic action which could be used for drug formulation.

KEY WORDS: phytochemical constituents, alkaloids, crude extracts and flavonoids.

INTRODUCTION

India is called Botanical Garden of the world and one of the worlds twelve leading biodiversity centers which contain over 45,000 different plant species, out of this, 15,000-20,000 species are with good medicinal properties of which only about 7,000-7,500 are being used by traditional practitioners. In India, it is estimated that there are about 25,000 licensed pharmacies of Indian system of medicine. Presently, about 1000 single drugs and about 3000 compound formulations are registered. Herbal industry in India uses about 8000 medicinal plants and the annual turnover of the Indian herbal medicinal industry is more lucrative. The Siddha system of medicine uses around 600, Ayurveda 700, Unani 700 and modern medicine about 30 plant species. After information technology, herbal technology is India's biggest revenue source.^[1]

Generally plants play an important role in medicinal properties for both preventive and curative. Phytochemicals are the plant derived substances have recently become great interest owing to their versatile application. Medicinal plants are richest bioresource of drugs in traditional system of medicine and it also

responsible for different colours, flavors and smell of plant. They also functions as medicaments. These medicinal values of plants lies in some chemically active substance, that produce a definite physiological action on the human body.^[2] There are thousands of species of medicinal plants used globally for the cure of different infections. These plants are used as antimicrobial agents and several works have been carried out by scientists to find out its scientific basis.^[3]

Since ancient times, people have been exploring the nature particularly plants in search of new drugs. This has resulted in the use of large number of medicinal plants with curative properties to treat various diseases. Nearly 80% of the worlds realizes on traditional medicines for primary health care, most of which involve the use of plant extracts. In India, almost 95% of the prescriptions were plant based in the traditional systems of Siddha, Unani, Ayurveda and Homeopathy. The study of plants continues principally for the discovery of novel secondary metabolites. Around 80% of products were of plant origin and their sales exceeded US \$65 billion in 2003.^[4] In India is varietal emporium of medicinal plants and is one of the richest countries in the world in regard

to genetic resources of medicinal plants. It exhibits a wide range in topography and climate, which has a bearing on its vegetation and floristic composition. Moreover, the agro-climatic conditions are conducive for introducing and domesticating new exotic plant varieties.^[5]

Limonia acidissima (L.) of family Rutaceae (Citrus family) belongs to the monotypic genus *Limonia*, confined to India, Pakistan, Sri Lanka and Southeast Asia.^[6] It is also known as woodapple, elephant-apple, monkey fruit, curd fruit, kath bel and kaitha. This plant is given as a medicine for the treatment of various disorders.^[7] Wood-apple is useful in preventing and curing scurvy and in relieving flatulence. Mashed seedless pulp of the raw fruit is beneficial in the treatment of dysentery, diarrhoea and piles. *L. acidissima*, considered to be a hepatoprotectant, possess different biological activities namely adaptogenic activity against blood impurities, leucorrhoea, dyspepsia and jaundice. Traditionally, all parts of the plants are given as natural medicine as a cure for various ailments.^[8] It is very often used against snakebites.^[9] People use it as a tonic for liver and heart, in diarrhoea and dysentery. This fruit is considered to be an effective treatment for hiccups and for problems of throat and gum.^[10]

The plant *Pergularia daemia* (Asclepiadaceae) known as “Veliparuthi” in Tamil, Uttaravaruni” in Sanskrit and “Utranajutuka” in Hindi. It is a hispid perennial herb that grows along the roadsides of India and other tropical and subtropical regions. Earlier reports indicate various beneficial uses of the herb, validating it as a medicinal herb. Traditionally the whole plant is used as anthelmintic, antipyretic, laxative and expectorant and to treat infantile diarrhea and malarial fever. The root of the plant is effective in treating convulsions, asthma, poisoning, mental disorder, anemia, leprosy and piles.^[11] Dried leaf of this plant is used as an emetic agent and is effective in treating bronchitis,^[12] asthma,^[13] rheumatic amenorrhea, dysmenorrhea,^[14] ^[15] wounds^[16] and to facilitate parturition. Fresh roots and shoots are found to be useful in treating whooping cough.^[17] The shoots of the plant are considered to be an effective agent for abortion.^[18] Stem bark is used in treating malaria^[19] and twig is effective as an antipyretic agent and serves as a good appetizer.^[20]

Phytochemical screening is very important in identifying new sources of therapeutically and industrially important compounds like alkaloids, flavonoids, phenolic compounds, saponins, steroids, tannins, terpenoids etc.^[21] The present study aimed to find the phytochemical constituents present in chloroform, ethyl acetate and methanol extracts different parts of *L. acidissima* and hexane, chloroform and methanol extracts of leaf of *P. daemia*.

MATERIALS AND METHODS

Collection and identification of Plant Material

Plants were collected from a place at Dornala, Prakasam District, Andhra Pradesh. Prakasam district is one of the Southernmost districts of Andhra Pradesh lies between 14°57' and 16°17' North latitude and 73°43' and 80°25' East longitude, occupying an area about 17,626 Sq. km. The Nallamalais and the Veligondla are the two major hill ranges in the district, of which Veramkonda situated in the Eastern Nallamalais has the highest peak (939 m). The Nallamalais hills which form a part of Eastern Ghats run through this district is distributed by several medicinal plants which are used traditionally by local tribal people.^[22] The total area of the district is 17,626 square kilometres. The total population of the district is 33; 84,192.

The authentication of the plant species were done by Prof. Dr. Vatsavaya S. Raju, Department of Botany, Kakatiya University, Warangal, Telangana-506 009, India. The plants *L. acidissima* and *P. daemia* were deposited in the Department of Botany, Kakatiya University and voucher specimens are identified as *Limonia acidissima* Groff - Acc.no. KUW1932 of Rutaceae and *Pergularia daemia* (Forssk.) Chiov. - Acc. no. KUW1926 of Apocynaceae (which now incl. Asclepiadaceae), All the collected plant parts were washed thrice with tap water and twice with distilled water to remove the adhering materials and other associated organisms.

Preparation of Plant Extracts

Selected parts of two plants were collected and left at room temperature for two weeks to dry. Samples were chopped into smaller pieces and then ground into powder. The samples were then stored in jars at room temperature until needed for extraction. Shade-dried medicinal plant samples were subjected for in 90% different organic solvents chloroform (60-62°C), ethyl acetate (76-77°C), and methanol (65°C) in a soxhlet apparatus (Borosil). After complete extraction, the filtrates were concentrated separately by rotary vacuum evaporation (>45°C) and then freeze dried (-20°C) to obtain solid residue. The extraction percentage was calculated by using the following formula:

$$\text{Percentage of extraction} = \frac{\text{Weight of the extract (g)}}{\text{Weight of the plant material (g)}} \times 100$$

These plant extracts were screened for the presence of phytochemical constituents by standard method. The plant extracts were dissolved in dimethyl sulphoxide (DMSO) and filtered through ‘millipore sterile filters’ (mesh 0.20 µm, Sartorius Stedim Biotech GmbH, Germany).

Phytochemical analysis

Chemical test were carried out using various extract such as chloroform, ethyl acetate and methanol. To identify the presence of phytochemical analysis of the extracts for

alkaloids, saponins, terpenoids and steroids, tannins, anthocyanidin, phenolic compounds, flavonoids, coumarins, quinones resins and glycosides was done by following the standard method according to Sofowara^[23] and Harborne.^[24]

Test for the Alkaloids

The extract was evaporated to dryness and the residue is dissolved in 1% HCL. To this solution was added Mayer's and Dragendorff's reagents. Appearance of any precipitate or turbidity indicates the presence of alkaloids.

Mayer's reagent: 1.3 g of HgCl₂ and 5 g of KI were dissolved separately in 60 ml of double distilled water respectively and both the solutions were mixed and diluted to 100 ml.

Dragendorff's reagent: 8 g of Bismuth nitrate was dissolved in 20 ml of concentrated HNO₃ and 27.2 g of KI in 50 ml of double dissolved water. Both the solutions were allowed to stand till KIO₃ crystallized out. Supernatant was decanted and final volume was adjusted to 100 ml.

Test for the Saponins

The plant extract is evaporated to dryness. Tap water was added and shaken vigorously. Formation of persistent foam of about 2 cm is taken as appositve reaction.

Test for the Terpenoids and Steroids

50 of H₂SO₄ is added along the sides of the test tube containing mixture of solvent HCl and anhydride. If there is any change in colour, from green to blue-green (sometimes via red or blue) indicates the presence of terpenoids and steroids.

Test for the Tannins

The plant extract is evaporated to dryness and the residue was dissolved in water and tested with 1% gelatine solution and gelatine salt solution (1g) gelatine dissolved in 10 g of NaCl (w/w) to separates volumes. The appearance of white precipitate will be regarded as positive reaction.

Test for the Anthocyanidin

To the plant extract was added equal volume of HCl. Appearance of red or purple colour indicates the presence of anthocyanides.

Test for Phenolic compounds

The formation of intense colour in the extract, in adding 1-2 drops of 1% ferric chloride to the extract is considered as a positive reaction test.

Test for the Flavonoids

Few ml of plant extract is added with conc HCl and Mg powder. The presence of flavonoids can be identified by the development of pink or magenta or red coloured foam.

Test for the Coumarins

To the plant extract, a few drops of alcoholic sodium hydroxide were added. Formation of yellow colour indicated the presence of coumarins.

Test for the Quinones

To 1 ml of plant extract, 1 ml of conc. H₂SO₄ was added. Formation of red colour shows the presence of Quinones.

Test for the Resins

Plant extract were treated with acetone. To this, small amount of water was added and shaken. The appearance of turbidity indicates the presence of resins.

Test for Glycosides

To the plant extract mixed with a little anthrone on a watch glass. Few drops of conc. H₂SO₄ was added and warmed gently over water bath. The presence of glycosides was identified by dark green colour formation.

To the plant extract few drops of glacial acetic acid, ferric chloride and conc. H₂SO₄ are added and observed for a reddish brown coloration at the junction of the two layers and the bluish green colour in upper layer.^[25]

RESULTS

The different plant extracts that were prepared from plants *L. acidissima* and *P. daemia* (Fig. 1 and Fig. 2) were subjected to phytochemical analysis for the presence of different bio-active compounds.



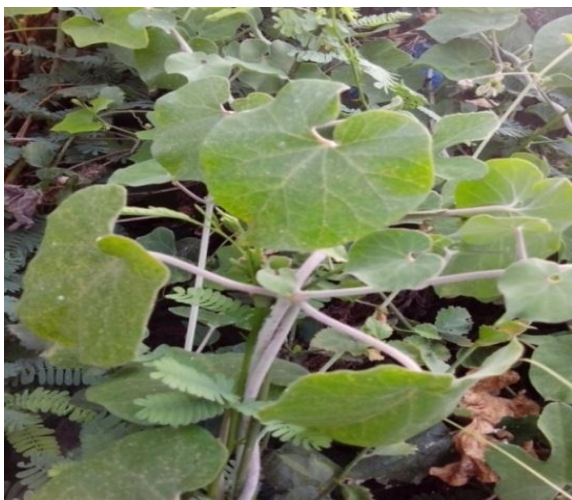


Fig. 1: *Limonia acidissima* (L.) plant Fig. 2: *Pergularia daemia* (L.) plant

The amount of extracts obtained from chloroform, ethyl acetate and methanol of leaf, stembark and root were 4.3 g, 6.5 g, 25 g, 4.5 g, 3.4 g, 10 g, 5 g, 6 g and 12 g respectively per 100 g of powder. More yield was obtained from methanolic leaf extract (25%) of *L. acidissima* (Table 1).

Table 1: Percentage yield of different crude extracts from *L. acidissima*.

Plant part	Solvent	Initial weight (g)	Yield of the extract (g)	Percentage of yield (%)
Leaf	Chloroform	100	4.3	4.3
	Ethyl Acetate	100	6.5	6.5
	Methanol	100	25	25
Stembark	Chloroform	100	4.5	4.5
	Ethyl Acetate	100	3.4	3.4
	Methanol	100	10	10.0
Root	Chloroform	100	5	5.0
	Ethyl Acetate	100	6	6.0
	Methanol	100	12	12.0

Table 2: Phytochemical investigation of *L. acidissima* in different extracts of Leaves, Stembark and Roots.

Phytochemical constituent	Leaves			Stembark			Roots		
	C	EA	M	C	EA	M	C	EA	M
Alkaloids	+	-	+	+	-	+	+	-	+
Saponins	-	-	+	-	-	-	-	-	-
Terpenoids and Steroids	-	+	+	-	-	-	-	-	-
Tannins	-	-	-	-	-	-	-	-	-
Anthocyanidins	-	-	-	-	-	-	-	-	-
Phenolic compounds	+	+	+	+	+	+	-	-	-
Flavonoids	+	+	-	-	-	-	-	-	-
Coumarins	-	-	-	-	-	-	+	+	+
Quinones	-	-	-	-	-	-	-	-	-
Resins	-	-	-	-	-	+	+	-	-
Glycosides	+	-	+	+	-	-	+	-	+

+ = Presence, - = Absence, C= Chloroform, EA= Ethyl acetate and M= Methanol

The amount of extracts obtained from hexane, chloroform and methanol of leaf were 15 g, 20 g and 25 g respectively per 100 g of powder. More yield was obtained from methanolic leaf extract (25%) of *P. daemia* (Table 3).

Phytochemical screening of the chloroform, ethyl acetate and methanolic extracts of *L. acidissima* leaves, stembark and roots have shown the presence of various medicinally active constituents (Table 2). A total of 11 phytochemicals were analysed. The chloroform extract of leaf showed the presence of four compounds, ethyl acetate extract showed the presence of three compounds and methanolic leaf extract of *L. acidissima* showed the presence of five phytochemicals. The stembark extract of chloroform showed the presence of three phytochemicals, ethyl acetate extract of stembark has shown the presence of one compound and methanolic extract of stembark shown the presence of three compounds. The root extract of chloroform showed the presence of four phytochemicals, ethyl acetate extract of root showed the presence of one compound and methanolic extract of root showed the presence of three compounds. Phenolic compounds were present in all solvents extracts of the leaf and stembark. But were present in all the crude extracts of root.

Phytochemical screening of the hexane, chloroform and methanolic leaf extracts of *P. daemia* showed the presence of various medicinally active constituents (Table 4). A total of 11 phytochemicals were analysed. The hexane extract of leaf showed the presence of one

compound, chloroform extract and methanol extract of leaf showed the presence of five compounds.

Table 3: Percentage yield of different crude extracts from *P. daemia* plant.

Plant part	Solvent	Initial weight (g)	Yield of the extract (g)	Percentage of yield (%)
Leaf	Hexane	100	20	20%
	Chloroform	100	20	20%
	Methanol	100	25	25%

Table 4: Phytochemical investigation of *P. daemia* leaf extraction.

Phytochemical constituent	Hexane extraction	Chloroform extraction	Methanol extraction
Alkaloids	-	+	+
Saponins	-	-	+
Terpenoids and Steroids	-	+	+
Tannins	-	+	+
Anthocyanidins	-	-	-
Phenolic compounds	-	-	-
Flavonoids	+	+	+
Coumarins	-	-	-
Quinones	-	-	-
Resins	-	-	-
Glycosides	-	+	-

DISCUSSION

Several studies have proved that the phyto-components present in a medicinal plant are widely responsible for the therapeutic potential of the plant. According to the World Health Organisation, medicinal plants would be the best source to obtain a variety of drugs.^[26]

The results of phytochemical screening showed that the chloroform extracts of *L. acidissima* contain alkaloids, phenolic compounds, flavonoids, coumarins, resins and glycosides. Ethyl acetate extracts of *L. acidissima* contain terpenoids and steroids, phenolic compounds, coumarins and quinines. Methanolic extracts of *L. acidissima* contain alkaloids, saponins, terpenoids and steroids, phenolic compounds, coumarins, resins and glycosides. The present work is correlation with Pranita et al.,^[27] who analyzed almost all the parts of *L. acidissima* which is rich in phytochemicals. Petroleum ether, chloroform, acetone and methanol extracts of root, bark and leaf have shown the presence of alkaloids. All the solvent extracts of root, bark and leaf show the presence of carbohydrates except petroleum ether extract of root and bark. Chloroform extract of bark contains higher amount of glycosides. Protein is present in almost all the non-polar and polar solvent extracts of root, bark and leaves but not in chloroform extract of root and acetone extract of bark and leaf. Saponins and phytosterols were found in chloroform, acetone and methanol extracts of root, bark, leaf and petroleum ether extract of leaf. Petroleum ether extract of root, bark, leaf and only methanolic extract of leaf possessed fixed oils and fats. Phenolic compound and flavonoids were detected in petroleum ether extracts of root and leaf, chloroform, acetone and methanolic extracts of root, bark and leaf. Only petroleum ether extract of root, bark and chloroform extract of leaf has shown the presence of gum and mucilages.

Similar study has done by Pratima et al.,^[28] who reported the phytochemical constituents identified in *L. acidissima*. The study revealed that, petroleum ether extracts of leaf, stem and fruit contains proteins, tannins, terpenoids and flavonoids, methanol and aqueous extracts of leaf, stem and fruit contains alkaloids, flavonoids, terpenoids, carbohydrates and proteins which is in correlation with our study.

Similar work was done by Attarde et al.,^[29] who did phytochemical screening of leaf of *Limonia acidissima* extracted in petroleum ether, chloroform and methanol. It was observed that these extracts have shown the presence of steroid, terpenoids, high amount of phenolic glycoside, tannins and flavonoids which is in corroboration with our findings.

Thomas and Ponnammal^[30] had studied all the parts of *L. acidissima* which are rich in secondary metabolites. Among the identified constituents, alkaloid was higher in bark and rind when compared to the other parts. Saponins, steroids and glycosides were present in all plant parts. Flavonoids are present only in pulp and seeds. Phenols were found only in bark and leaves. Gum and mucilage is present in all the plant parts tested except leaf. Leaf, pulp and seed possessed fixed oils and fats. Resins are present only in pulp. Bark, leaf and seeds showed the presence of tannins. The study supports the present findings that leaf and stem bark consists of alkaloids, saponins, terpenoids and steroids, phenolic compounds, flavonoids, resins and glycosides.

The phytochemical screening of leaf of *P. daemia* shown the presence of favonoids in hexane extract; alkaloids, terpenoids and steroids, tannins, flavonoids and glycosides in chloroform extract; alkaloids, saponins, terpenoids and steroids, tannins and flavonoids in

methanol extract. So the present work is supported by Karthishwaran^[31] where methanolic extract of the leaf of *P. daemia* showed the presence of flavonoids, steroids, carbohydrates, alkaloids, tannins and terpenoids.

Smilarly Sridevi *et al.*,^[32] observed the presence flavonoid, tannins, alkaloids, glycosides, terpenoids, steroids and carbohydrates in ethanolic extracts of aerial parts (leaf, stem and flower) of *P. daemia* which confirms the present work.

Pravin and Akkewar^[33] did the qualitative analysis of hexane extract leaf of *P. daemia* which has shown the presence of flavonoids which is in agreement with the present study. The chloroform extract of leaf shown the presence of glycosides, flavonoids, terpenoids, cardiac glycosides whereas in our investigation alkaloids were also observed. The methnolic extract of leaf shown the presence of alkaloids, tannins, reducing sugars, saponins and phlobatannins but in our study we observed the presence of alkaloids, saponins, terpenoids and steroids, tannins and flavonoids.

Madhuri and Navnath^[34] studied for phytochemical constituents in chloroform extract and methanol extract of stem *P. daemia*. The chloroform of stem has shown the presence of tannins, betacyanins and alkaloids but our study shown the presence of terpenoids and steroids, flavonoids, glycosides along with alkaloids and tannins. The methanol extract of stem consists of tannins, flavonoids, quinones, betacyanins, alkaloids, glycosides, terpenoids and phenols whereas in the present study we observed the presence of saponins along with tannins, flavonoids, quinones, betacyanins, alkaloids, glycosides, terpenoids and phenols.

Thus our study is in correlation with the peer researchers and our results confirm the earlier work. Hence, the plants *L. acidissima* and *P. daemia* have various secondary metabolites which indicates the therapeutic potential for different ailments.

CONCLUSION

Mostly plants play a major role in traditional medicinal system to combat several diseases. Generally plants have many phytochemicals like alkaloids, saponins, terpenoids and steroids, tannins, anthocyanidin, phenolic compounds, flavonoids, coumarins, quinones resins and glycosides with specialized properties. The plants screened for phytochemical analysis seemed to have the potential to act as a source of useful drugs and also to improve the health status of consumers that were assayed. This study justifies the use of plant species in medicinal field.

ACKNOWLEDGEMENT

I am thankful to the Co-ordinator, Department of Zoology and Aquaculture, Acharya Nagarjuna University, Guntur, Andhra Pradesh for providing necessary facilities. I am highly thankful to the

authorities of UGC, New Delhi for providing me financial assistance in the form of UGC-BSR fellowship.

REFERENCES

1. Sharma A, Shanker C, Tyagi L, Singh M, Rao CV. Herbal medicine for market potential in India: An Overview. *Acad J Plant Sci*, 2008; 1(2): 26-36.
2. Karunyadevi S, Arun N, Surekha V. Screening of phytochemical compounds, antioxidant and antimicrobial activity of *Aloe vera* and Arkaa. *Advanced Biotech*, 2009; 9: 38-43.
3. Omotayo AE. Antibacterial activity of some antimalarial plants. *Proceedings of Nigerian Society for Microbiology*, 1998; 39: 69-72.
4. Bhushan Patwardhan, Ashok DB. Vaidya, Mukund Chorghade. *Ayurveda and natural products discovery*. *Current Science*, 2004; 86(6): 789-799.
5. Martins AP, Salgueiro L, Goncalves MJ, Proença da Cunha A, Vila R, Cañigüeral S, Mazzoni V, Tomi F, Casanova J. Essential oil composition and antimicrobial activity of three *Zingiberaceae* from S Tome e Principe. *Planta Med*, 2001; 67(6): 580-584.
6. Allen BM. *Malayan Fruits-An introduction to cultivated species*. Donald Moore Press Ltd. Singapore, 1967; 245.
7. Khare, Chandrama P. *Indian medicinal plants: an illustrated dictionary*. Springer Science & Business Media, 2008.
8. Morton JF. *Wood-Apple*. In: *Fruits of warm climates*, Flare Books, Miami, Florida, 1987; 190-191.
9. Yusuf M, Chowdhury JU, Wahab MA, Begum J. *Medicinal plants of Bangladesh*. Bangladesh Council of Scientific and Industrial Research, 1994; 192.
10. Ratnayake, RMRNK, Sumithra, HJ, Fernando MD, Palipane KB. Effect of GRAS compounds on *Aspergillus* rot of wood-apple (*Feronia limonia*). *Phytoparasitica*, 2009; 37(5): 431-436.
11. Yoganarasimhan SN. "Medicinal plants of India. Volume 2. Tamil Nadu." Bangalore: The Author i 2000.
12. Mittal OP, Tammz C, Reichstein T. Glycosides and aglycons. CCXXVII. The glycosides of *Pergularia extensa*. *Helv Chim Acta*, 1962; 45: 907.
13. Elango V, Ambujavalli L, Amala Basker E, Sulochana N. Pharmacological and microbiological studies on *Pergularia extensa*. *Fitoterapia*, 1985; 56(5): 300-302.
14. De laszlo H, Henshaw PS. Plant materials used by primitive peoples to affect fertility. *Science*; 1954; 119: 626-631.
15. Dutta A, Ghosh S. *Daemia extensa*. *Indian J Pharm*, 1947b; 9: 58-60.
16. Pushpangadan P, Atal CK. Ethno-medico-botanical investigations in Kerala I. Some primitive tribals of Western Ghats and their herbal medicine. *J Ethnopharmacol*, 1984; 11(1): 59-77.

17. Kokwaro JO. A review of research on plants for fertility regulation in Africa. Proc who symposium on plant -derived products for fertility regulation. 1981; 12(3): 149-152.
18. Reddy MB, Reddy KR, Reddy MN. A Survey of plant crude drugs of Anantapur district, Andhra Pradesh, India. Int J Crude Drug Res, 1989; 27(3): 145-155.
19. Kohler I, Jenett Siems K, Kraft C, Siems K, Abbiw D, Bienzle U, Eich E. Herbal remedies traditionally used against malaria in Ghana: Bioassay-guided fractionation of *Microglossa pyridolia* (asteraceae). Z Nature Forsch Ser C, 2002; 57C (11/12): 1022-1027.
20. Gill LS, Akinwumi C. Nigerian folk medicine: Practices and beliefs of the Ondo people. J Ethnopharmacol. 1986; 18(3): 257-266.
21. Akindele AJ, Adeyemi OO, Anti-inflammatory activity of the aqueous leaf extract of *Byrosocarpus coccineus*. Fitoterapia, 2007; 78(1): 25-28.
22. R. Krishna Mohan, PV Bhirava Murthy. Plants used in traditional medicine by tribals of Prakasam district, Andhra Pradesh, Anc Sci life, 1992; 11(3-4): 176.
23. Sofowara A. Medicinal plants and traditional medicine in Africa, Spectrum Books Ltd, Nigeria. 1993; 289.
24. Harborne JB. Phytochemical methods. Chapman & Hall Ltd, London. 1973; 49-188.
25. Siddiqui AA, Ali M. Practical Pharmaceutical Chemistry; 1st ed.; CBS Publishers and Distributors, New Delhi, (1997); 126-131.
26. Arunkumar, S., Muthuselvam. Analysis of phytochemical constituents and antimicrobial activities of *Aloe vera* L. Against clinical pathogens. World J Agril Sci, 2009; 5(5): 572-576.
27. Pranita P Shuddhalwar, Vijay N Charde, Suvarna P Patil. Study of phytochemicals in Non-Polar and Polar Solvent Extracts of Root, Bark and Leaf of *Limonia acidissima* and their Anti-bacterial Efficacy. Int J Res BioSci Agril Tech, online special issue. 2014.
28. Pratima Vijayvargia, Soniya Choudhary and Rekha Vijayvergia. Preliminary phytochemical screening of *Limonia acidissima* linn. Int J Pharm Pharm Sci, 2014; 6(1): 134-136.
29. Attarde DL, Chaudhari BJ, Bhambar RS. Phytochemical investigation and *in vitro* antioxidant activity of extracts from leaves of *Limonia acidissima* linn. (Rutaceae), J Pharm Res, 2011; 4(3): 766-768.
30. Asha Thomas, NR Ponnammal. Preliminary studies on phytochemical and antibacterial activity of *Limonia acidissimal*. plant parts. Anc Sci Life, 2005; 25(2): 57-61.
31. Karthishwaran, S Mirunalini, G Dhamodharan, M Krishnaveni, V Arulmozhi. Phytochemical investigation of methanolic extract of the leaves of *Pergularia daemia*. J Biol sci, 2010; 10(3): 242-246.
32. Sridevi G, Prema Sembulingam, Sekar Suresh, Sembulingam K. Phytochemical Analysis of *Pergularia Daemia* for Its Bioactive Components through Gas Chromatographic Mass Spectrometry (GCMS). IOSR J Pharm, 2014; 4(5): 41-46.
33. Pravin S Jogi, Dr. DM Akkewar. Phytochemical screening and antimicrobial activity of medicinal plant *Pergularia daemia* From Chandrapur Forest Region. Int J Nat Prod Res, 2012; 1(3): 61-63
34. Madhuri M Deshmukh, Navnath G Kashid. Phytochemical analysis and antimicrobial activity of *Pergularia daemia* (Forsk) Chiov, against multidrug resistance human pathogenic bacteria. World J Pharm Res, 2018; 7(4): 1085-1094.