

PHYTOCHEMICAL SCREENING OF ETHANOLIC EXTRACTS OF *BARLERIA PRIONITIS* LINN AND *PHYLLANTHUS ACIDUS*

Boopathi T.*

Department of Pharmacology, Sri Shanmugha College of Pharmacy, Salem.

Article Received on
25 April 2023,

Revised on 14 May 2023,
Accepted on 04 June 2023

DOI: 10.20959/wjpr202310-28031

*Corresponding Author

Boopathi T.

Department of
Pharmacology, Sri
Shanmugha College of
Pharmacy, Salem.

ABSTRACT

In this study, we assessed phytochemical screening of extracts from *Barleria prionitis* linn and *Phyllanthus acidus*. Qualitative phytochemical screening of *Barleria prionitis* revealed the presence of bioactive compounds such as carbohydrates, glycosides, alkaloids, tannins, flavonoids, phenols and saponins in ethanolic extract. On the other hand the ethanolic extract of *Phyllanthus acidus* showed the presence of glycosides, alkaloids, flavonoids, steroids, phenols.

KEYWORDS: Phytoconstituents, *Barleria prionitis*, *Phyllanthus acidus*, Ethanolic extracts.

1. INTRODUCTION

In India medicinal plants and traditional medicine have been the basis of traditional healthcare especially in remote area where modern health facilities are inadequate. India being a largest producer of medicinal plants is rightly called the botanical garden of the world. Ayurveda, the system of medicine indigenous of India has thousands years of history with all its pride and glory. Ayurvedic pharmacopoeia comprises of both vegetable and mineral products. (Mukherjee, 2002) India is one of the world's leading biodiversity center with the presence of over 45000 different plant species. India has 15000-18000 species of flowering plants, 2500 algae, 23000 Fungi, 1600 types of lichens and 1800 varieties of bryophytes and estimated 30 million types of microorganisms. These about 15000-20000 plants have good medicinal value. However, only traditional communities use about 7000-7500 for their medicinal values. Plant chemistry has been the basis for many synthetic drugs in modern pharmacopoeia (Chaudhri, 1996).

1.1 Importance of herbal medicine in health care system

Herbal medicines are prepared from a variety of plant material such as leaves, stems, roots, bark, etc. They usually contain many biologically active ingredients and are used primarily for treating mild or chronic ailments. Herbal remedies can also be purchased in the form of pills, capsules or powders, or in more concentrated liquid forms called extracts and tinctures. They can apply topically in creams or ointments, soaked into cloths and used as compresses, or applied directly to the skin as poultice (Kokate CK., 1994).

1.2 Difference of Herbal and Conventional Drugs

Compared with well-defined synthetic drugs, herbal medicines exhibit some marked differences, namely.

1. The active principles are frequently unknown standardization, stability and quality control are feasible but not easy;
2. The availability and quality of raw materials are frequently problematic.
3. Well-controlled double-blind clinical and toxicological studies to prove their efficacy and safety are rare.
4. Empirical use in folk medicine is a very important characteristic.
5. They have a wide range of therapeutic use and are suitable for chronic treatments.
6. The occurrence of undesirable side effects seems to be less frequent with herbal medicines, but well-controlled randomized clinical trials have revealed that they also exist.
7. They usually cost less than synthetic drugs (Gesler WM., 1992).

1.3 Problems with modern Drugs

1. High cost and long time taken in development of a new drug.
2. Toxicity
3. Non-renewable source of basic raw materials.
4. Environmental pollution by the chemical industry.
5. Inadequate, especially in management of certain chronic diseases. (Trease and Evans, 1989).

2. LITERATURE REVIEW

2.1 LITERATURE REVIEW OF *Barleria prionitis*

1. Piush Sharma, et al., (2013) investigated and reported the phytochemical and ethnomedical values of *Barleriapronitis* L. (Acanthaceae) which have been established against various disease models using modern scientific methodologies and tools.
2. Nidhi et al., (2013) evaluated the antibacterial activity of rhizome of *Barleria prionitis* in methanol extract.
3. Manider Karan et al., (2013) assessed topical anti-inflammatory activity of *Barleria prionitis* and *Barleria Cristata* against croton oil induced ear oedema in female rats.
4. Kuldeep Singh et al., (2013) evaluated the anti-inflammatory effect of methanol extract of *Barleriapronitis* on carragennan induced rat paw oedema.
5. Manjusha et al., (2013) evaluated the gastro protective activity of methanolic extract of *Barleria prionitis* on ethanol and indomethacin induced ulcer models in rats.
6. Swathi Paul. et al., (2012) reported the potent antibacterial activity of different leaf extracts of *Barleriaproinitis* L.
7. ReemaDheer et al., (2011) studied the antibiotic activity of alcoholic extract of leaf and root of *Barleria prionitis* in normal and alloxan induced diabetic rats.
8. Chavan Chetan et al., (2011) investigated the antioxidant activity of ethanolic and aqueous extract of whole plant of *Barleria prionitis*.
9. Shabir A. Lone et al., (2011) reported the effect of different plant growth regulators on in-vitro propagation of *barleriapronitis*linn.

2.2 LITERATURE REVIEW OF *Phyllanthus acidus*

1. M. Suriyavathana et al., (2011) reported the proximate analysis on biochemical study of *Phyllanthus acidus*, *Phyllanthus emblica* and *Citrus limon*.
2. R.C. Jagessar et al., (2008) reported the Selective Antimicrobial properties of *Phyllanthus acidus* leaf extract against *Candida albicans*, *Escherichia coli* and *Staphylococcus aureus* using Stokes Disc diffusion, Well diffusion, Streak plate and a dilution method.
3. A.Jagajothi et al., (2013) evaluated the antimicrobial Activity and phytochemical analysis of *Phyllanthus acidus*.
4. Raja Chakraborty et al., (2012) investigated the anti-inflammatory, antinociceptive and antioxidant activities of *Phyllanthus acidus* L. Extracts.
5. Ramachandra Y L et al., (2014) assessed the antioxidant potential of *Phyllanthus acidus* bark extracts.

6. Md. Razibul Habib et al., (2011) reported the evaluation of antioxidant, cytotoxic, antibacterial potential and photochemical screening of chloroform extract of *Phyllanthus acidus*.
7. Nilesh Kumar Jain et al., (2011) investigated the Protective effects of *Phyllanthus acidus* (L.) Skeels leaf extracts on Acetaminophen and thioacetamide induced hepatic injuries in Wistar rats.
8. Md. Mominur Rahmana et al., (2011) reported the antibacterial, cytotoxic and antioxidant potential of Methanolic extract of *Phyllanthus acidus*.
9. Jorge A. Pino et al., (2008) evaluated the volatile compounds of grosella (*Phyllanthus acidus* [L.] Skeels) fruit.

3. AIM AND PLAN OF WORK

3.1 AIM OF WORK

The present study was conducted to compare the phytochemical screening from two medicinal plants *Barleria prionitis* and *Phyllanthus acidus*.

3.2 PLAN OF WORK

1. Selection of Plants.
2. Collection and authentication of plants.
3. Extraction of leaves of *Barleria prionitis* and *Phyllanthus acidus*.
4. Qualitative phytochemical studies.

4. PLANT PROFILE

4.1 PLANT PROFILE OF *BARLERIA PRIONITIS*



General Information of *Barleria prionitis* linn

A genus of herbs belongs to the family *acanthaceae* well distributed in India, Ceylon, Southasia etc.

LATIN BINOMIAL & VERNACULAR NAMES

Source: *Berlaria prionitis* linn.

Family: Acanthaceae

VERNACULAR NAMES

English Name - Percupine flower

Tamil Name - Chemmulli, Korantam, Sullimalar

Sanskrit - Artagalaha, Bana, Jhinti

Malayalam - Karimkurunni

Kannada - Mullugoranta

Hindi Name - Jhinti, Vajradanti, Parush

Marathi - Kolita, Pivalikorantiu

Gujarati – Pilokantasheriyo

SCIENTIFIC CLASSIFICATION

Kingdom - Plantae

Subkingdom - Tracheobionta

Division - Mangoliophyta

Subdivision - Spermatophytina

Class - Mangoliopsida

Subclass –Asteridae

Order - Lamiales

Super order - Asteranae

Family - Acanthaceae

Genus - Berlaria

Species – Prionitis

Chemical constituents

Barleriaprimonitis consist of acabarlerin, barlerin, β - sitosterol, flavanol, glycoside, iridoids and scutellarein-7-neohesperidoside.

Aerial parts of *Barleria prionitis* consist of phenyl ethanol glycoside, barlerinoside along with six iridoid glycosides, shanzhiside methyl ester, 6-o-trans-p-coumaroyl-8-o-acetyl shanzhiside methyl ester, barlerin, acetyl barlerin, 7-methoxy diderroside, luplinoside. Also prinoside A, prinoside B, prinoside C.

Traditional uses

Traditionally the plant is used for asthma, whooping cough and the leaves and roots are used against toothache, rheumatism, cataract, leucoderma, scabies, liver ailments, piles treatment, diuretic, ulcers and also in cut, wounds, malaria, fever and irritation control.

Pharmacological activity

Anti-fertility activity, anti-oxidant activity, anti-inflammatory activity, hepatoprotective activity, anti-microbial activity, anthelmintic, anti dental decay activity, anti-diabetic activity, anti-diarrhoeal activity, diuretic activity, besides these it has got AChE inhibitory activity, anxiolytics. (Banerjee D., 2012)

4.2 PLANT PROFILE OF PHYLLANTHUS ACIDUS**General Information of *Phyllanthus acidus***

A genus of herbs belongs to the family phyllanthaceae well distributed in throughout Asia and also in the Caribbean region, Central and South America etc.

LATIN BINOMIAL & VERNACULAR NAMES

Source :*Phyllanthusacidus*.

Family :Euphorbiaceae (or) Phyllanthaceae

VERNACULAR NAMES

English Name - Goos-berry

Tamil Name - Araineelikai

Sanskrit - Lavali, Sugandhamula

Malayalam - Arinelli, Nellikkapuli

Kannada - Kirnelli, Nallikai

Hindi Name - Chillimilli

Marathi - Rayawala

Gujarati - KhaatiAawala, Raayaamali

SCIENTIFIC CLASSIFICATION

Kingdom - Plantae

Subkingdom - Tracheobionta

Division - Mangoliophyta

Class - Mangoliopsida

Subclass –Rosidae

Order - Lamiales

Super order - Malpighiales

Family - Euphorbiaceae or Phyllanthaceae

Genus - Phyllanthus

Species – Phyllanthusacidus

Parts used

Leaves and seeds

Phytoconstituents

The important chemical constituents present in the *Phyllanthus acidus* such as lignin, terpenes, sterols, polyphenolic compounds, tannins, flavanoids, glycosides and alkaloids. The root bark contains saponins, gallic acid, tannins and a crystalline substance, lupeol, 4-hydroxybenzoic acid, caffeic acid, adenosine, kaempferol and hypogallic acid.

Medicinal use

In India, the fruit are taken as liver tonic, to enrich the blood. The syrup is prescribed as stomachic and the seeds are cathartic. The leaves, with added pepper, are poulticed on sciatica, lumbago, rheumatism. The acrid latex of various parts of the tree is emetic and purgative. (Siow-Ping Tan., 2020)

5. MATERIALS AND METHODS

5.1 COLLECTION AND AUTHENTICATION OF PLANT

The leaves of *Barleria prionitis* L and *Phyllanthus acidus* L were collected from the surrounding areas of Erode district, Tamilnadu, India. The samples were identified and

authenticated (Reg.No: BSI/SRC/5/23/2022/Tech/583 and 587) at Botanical Survey of India, Southern circle, Coimbatore.

Extraction procedure

The leaves were dried under shade and then made in to a coarse powder with a mechanical grinder. Powdered dried leaves were passed through sieve No. 40 and stored in an airtight container for further use. Both petroleum ether and ethanol extraction were carried out by using Soxhlet extractor.

1) Preparation of petroleum ether extract

The dried powder material of leaves (100gm) were first extracted with petroleum ether (60-80°) in a Soxhlet apparatus and after complete extraction (48 hrs), the solvent was removed by distillation under reduced pressure and resulting semisolid mass was vacuum dried using rotary flash evaporator.

2) Preparation of ethanolic extract

After the extraction with petroleum ether the same plant material were dried and again extracted with ethanol (99.9% v/v) in Soxhlet apparatus and after complete extraction (48 hr) the solvent was removed by distillation under reduced pressure and resulting semisolid mass was vacuum dried using rotary flash evaporator. All the extracts were subjected to various chemical tests to detect the presence of different phytoconstituents.

5.2 PHYTOCHEMICAL SCREENING OF *Barleria prionitis* AND *Phyllanthus acidus*

The plant extracts were subjected to preliminary phytochemical screening for the detection of various plant constituents present. The term qualitative analysis refers to establishing and proving the identity of a substance. The active ingredients, after isolation, can be incorporated into the modern medicine for the development of newer formulation for therapeutic ailments.

Qualitative phytochemical analysis

The petroleum ether and ethanolic extracts of *Barleria prionitis* and *Phyllanthus acidus* were subjected to qualitative tests for the detection of various plant constituents (Kokate CK.), (Krishnaswamy NR.), (Surekhaayarnalkar.), (Khandelwal KR.), (Rajgopal G.).

1. DETECTION OF CARBOHYDRATE

Small quantity of extract was dissolved in 4ml of distilled water and filtered. The filtrate was collected and subjected for the following tests.

a. Molisch's test: 1 ml of filtrate was treated with 2-3 drops of 1% alcoholic α -naphthol solution and 2 ml of conc. sulphuric acid was added along the sides of the test tube. Appearance of brown to violet ring, indicate the presence of carbohydrate.

b. Fehling's test: To the Fehling solution A and B extract was added and boiled. The formation of brick red precipitate indicates the presence of reducing sugar.

c. Benedict's test: 1 ml of extract was added to 5ml of Benedict's reagent, was added and boiled for 2 mins and cool. Formation of a red precipitate shows the presence of sugars.

d. Tollen's test: 1ml of extract was added with 2ml of Tollen's reagent was added and boiled. A silver mirror is obtained inside the wall of the tube which indicates the presence of aldose sugar.

2. TEST FOR GLYCOSIDES

a. Legal's test

The filtrate was hydrolyzed with dilute hydrochloric acid and heated on water bath. Then added 1ml of pyridine and few drops of sodium nitroprusside solution, made alkaline with sodium hydroxide solution. Appearance of pink to red colour shows the presence of glycosides.

b. Borntrager's test: Filtrate was hydrolyzed with dilute hydrochloric acid on water bath, then treated with chloroform and shake well. After that separates the chloroform layer and added equal volume of dilute ammonia solution. If ammonia layer acquire pink or violet colour, indicates the presence of glycosides.

c. Baljet test: 1ml of extract was added to 1ml of sodium picrate solution and the yellow to orange colour shows the presence of glycosides.

d. Keller killiani test: The ethanol extract 0.5ml of strong solution of lead acetate was added and filtered. The filtrate is shaken with 5ml of chloroform. The chloroform layer is separated in a porcelain dish and removes the solvent by gentle evaporation. Dissolve the cool residue in 3ml of glacial acetic acid containing 2 drops of ferric chloride solution carefully transferred the solution to the surface of 2ml of concentrated sulphuric acid. A reddish brown layer form at the junction of the 2 liquid and the upper layer slowly becomes bluish green, darkening the withstanding.

3. DETECTION OF ALKALOIDS

Small quantity of extract was treated with few drops of dilute hydrochloric acid and filtered it. The filtrate was collected and subjected for tests with following reagents.

a. Mayer's reagent: To the filtrate potassium mercuric iodide was added. The formation of cream colour precipitate, it shows the presence of alkaloids.

b. Dragendroff's reagent: To the filtrate potassium bismuth iodide was added. If it shows reddish brown precipitate, indicates the presence of alkaloids.

c. Wagner's reagent: To the filtrate iodine in potassium iodide solution was added. If it shows reddish brown precipitate, indicates the presence of alkaloids.

d. Hager's reagent: To the filtrate saturated aqueous solution of picric acid was added. Formation of yellow precipitate indicates the presence of alkaloids.

e. Tannic acid test: 1ml of extract was added to 1ml of 10% tannic acid solution, buff coloured indicates the presence of alkaloids.

4. DETECTION OF PHYTOSTEROL AND STEROIDS

Small quantity of extract was dissolved in 5ml of chloroform and then subjected to the following tests.

a. Salkowski test: To the above solution 1 ml chloroform and few drops of concentrated sulphuric acid was added. The test tube was shaken for few minutes. The development of red colour in chloroform layer indicates the presence of steroids.

b. Liebermann- Burchard reaction: To the above solution 1 ml of chloroform and few drops of concentrated sulphuric acid and 1-2 ml of acetic anhydride were added. Development of red colour first, then blue and finally green colour, indicates the presence of steroids.

c. Liebermann's reaction: To 3 ml of extract in a test tube, 1 ml of acetic anhydride was added and gently heated. The contents of test tube were cooled. Few drops of concentrated sulphuric acid was added from the side of test tube. Appearance of blue shows the presence of steroids.

5. DETECTION OF PROTEINS AND AMINOACIDS

Small quantity of the extract was dissolved in few ml of water and filtered. The collected filtrate was used for following tests.

a. Millon's test: To the filtrate Millon's reagent was added. If white precipitate slowly turns to red colour, indicates the presence of proteins.

b. Biuret test: Filtrate was treated with 5% sodium hydroxide and few drops of 1% copper sulphate solution. Formation of violet or pink colour indicates the presence of proteins.

c. Ninhydrin test: To the filtrate Ninhydrin reagent was added. Development of violet or purple colour indicates the presence of amino acids.

d. Xanthoproteic test: 1ml of extract was treated with 1ml of concentrated nitric acid. A white precipitate formed, it was boiled and cooled. Then 20% of sodium hydroxide or ammonia is added. Orange colour indicates the presence of aminoacid.

6. DETECTION OF TANNINS

The test extract was dissolved in water, warmed and filtered. The filtrate was used for the following tests.

a. Ferric chloride test: 5 ml of filtrate was allowed to react with 1 ml of 5% ferric chloride solution. If dark green or deep blue colour is obtained, tannins are present.

b. Lead acetate test: 5 ml of filtrate was treated with 1 ml of 10% lead acetate solution. Yellow colour precipitation, indicates the presence of tannins.

c. Potassium dichromate test: 5 ml of filtrate was treated with 1 ml 10% aqueous potassium dichromate solution. If yellowish-brown precipitate formed it suggest the presence of tannins.

d. Gelatin solution test: 1% w/v solution of gelatin in water, containing 10% sodium chloride was prepared. A little of this solution was added to the filtrate. If white precipitate is obtained, tannins are present.

e. Vanillin hydrochloride test: 1ml of extract was added with vanillin hydrochloride. Formation of purplish red colour indicates the presence of tannins.

7. DETECTION OF FLAVONOIDS

a. Shinoda test: The small quantity of extract was dissolved in alcohol, to that piece of magnesium followed by concentrated hydrochloric acid were added by drop wise and heated. Appearance of magenta colour shows the presence of flavonoids.

b. Pew's test: A pinch of zinc powder and about 5 drops of 5N hydrochloric acid were added to the test solution. The formation of deep purple red or cherry red colour indicates the presence of flavonoids.

c. Extract was treated with aqueous sodium hydroxide solution. Development of yellow colour indicates the presence of flavonoids.

d. Extract was treated with sulphuric acid. Development of yellow to crimson shows the presence of flavonoids.

8. DETECTION OF SAPONINS

- a. Foam test:** The extract was diluted with distilled water to 20 ml and shaken in a graduated cylinder for 15 minutes. Development of stable foam suggests the presence of saponins.
- b. Heamolytic test:** To the extract or dry powder added one drop of blood placed on glass slide. If heamolytic zone appears shows the presence of saponins.
- c. Lead acetate test:** 1ml of sample solution was treated with 1% of lead acetate solution, formation of a white precipitate indicates the presence of saponins.

9. DETECTION OF TERPENOIDS

- a. Knoller's Test:** In a test tube, 2 or 3 granules of tin was added and dissolved in 2 ml of thionyl chloride solution. Then, test solution was added. A deep purple colour that changes to red indicates the presence of terpenoids.
- b. Salkowsky's Test:** 5ml of extract was mixed in 2ml of chloroform and concentrated sulphuric acid was carefully added to form a layer. Formation of reddish brown coloration of the interface indicates the presence of terpenoids.

10. TEST FOR FIXED OIL AND FATS

- a. Spot test:** Pressed a small quantity of extract between the two filter papers, the strain on the filter paper indicates the presence of fixed oils.
- b. Saponification test:** Added a few drops of 0.5N of alcoholic potassium hydroxide to small quantity of various extract along with a drop of phenolphthalein separately and heat on a water bath for 1 to 2 hours. The formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats.

11. TEST FOR GUMS AND MUCILAGE

10ml of ethanol extract was slowly added 25 ml of absolute alcohol with constant stirring filter the precipitate and dried in air. The precipitate for its swelling property indicates the presence of carbohydrates.

6. RESULTS AND DISCUSSION

The leaves of *Barleria prionitis* and *Phyllanthus acidus* were selected for the detailed study to evaluate its phytochemical and biological properties. We have taken its detailed phytochemical investigations to prove its appropriate identification and rationalize its use as drug of therapeutic importance.

6.1 QUALITATIVE PHYTOCHEMICAL ANALYSIS

In the phytochemical studies, petroleum ether, ethanolic extracts of leaves of *Barleria prionitis* and *Phyllanthus acidus* showed the presence various phytoconstituents.

Preliminary phytochemical screening of *Barleria prionitis* revealed the presence of bioactive compounds such as glycosides, alkaloids, tannins, flavonoids, phenols and saponins in ethanolic extract and fixed oil, gums and mucilage in petroleum ether extract. On the other hand the petroleum ether extract of *Phyllanthus acidus* contained fixed oil, fats, gums and mucilage. While the ethanolic extract showed the presence of glycosides, alkaloids, flavonoids, steroids and phenols. Different phytochemicals have been found to possess a wide range of activities, which may help in protection against chronic diseases. The results are given in Table-1.

Table 1: Data showing the preliminary phytochemical screening of the leaves extracts of *Barleria prionitis* and *Phyllanthus acidus*.

Phytoconstituents	Phyllanthusacidus		Barleriaprimonitis	
	Petroleum Ether	Ethanol (99.9%v/v)	Petroleum Ether	Ethanol (99.9%v/v)
Carbohydrate	Absent	Absent	Absent	Present
Glycosides	Absent	Present	Absent	Present
Alkaloids	Absent	Present	Present	Present
Steroids	Absent	Present	Present	Absent
Tannins	Absent	Absent	Absent	Present
Flavonoids	Absent	Present	Absent	Present
Phenols	Absent	Present	Absent	Present
Saponins	Absent	Absent	Absent	Present
Terpenoids	Absent	Absent	Absent	Present
Fixedoilandfats	Present	Absent	Present	Absent
Gumsand mucilage	Present	Absent	Present	Absent

7. SUMMARYAND CONCLUSION

The term medicinal plants include plants have a medicinal activities. Medicinal plants used in drug development and synthesis, because of its rich resource of ingredients. There are about half million plants around the world and they have medicinal properties and it could be decisive in the treatment of future or present studies. In developed countries, plant drug constituent as much as 25% of the total drugs, while in fast developing countries, the contribution is as much as 80%. The economic importance of medicinal plants is much more to countries such as India than to the rest of the world. Only a relatively small fraction of the world's green pharmacy has been investigated for possible pharmacological and therapeutic

properties. This holds the promise of investigation important phytoconstituents from plant origin.

The leaves of *Barleria prionitis* and *Phyllanthus acidus* were selected for the detailed study to evaluate its phytochemical properties. We have taken its detailed phytochemical investigations to prove its appropriate identification and rationalize its use as drug of therapeutic importance.

The plant materials were collected from the surrounding areas of Erode district and authenticated. The leaves were powdered and extracted with petroleum ether and absolute ethanol using Soxhlet apparatus separately. The extracts were concentrated to dryness under reduced pressure and controlled temperature. Qualitative phytochemical screening was carried out to identify the phytoconstituents.

Preliminary phytochemical screening of *Barleria prionitis* revealed the presence of bioactive compounds such as carbohydrates, glycosides, alkaloids, tannins, flavonoids, phenols and saponins in ethanolic extract. On the other hand the ethanolic extract of *Phyllanthus acidus* showed the presence of glycosides, alkaloids, flavonoids, steroids, phenols.

The present results will form the basis for selection of plant species for further investigation in the potential discovery of new natural bioactive compounds. Further studies which aimed at the isolation and structure elucidation of antibacterial active constituents from the plant have been initiated.

BIBLIOGRAPHY

1. Anonymous. British National Formulary. Publication of British Medical Association, UK. 2004.
2. Brower V., Nat Biotechnol, 1998; 16: 728–731.
3. Brown R.G., Toxicity of Chinese herbal remedies. Lancet, 1992; 340: 673.
4. Banerjee D., Maji A.K., Mahapatra S., Barleriapronitis Linn: A review of its traditional uses, phytochemistry, pharmacology and toxicology. Research Journal of Phytochemistry, 2012; 6(2): 31-41.
5. Calixto JB., Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phytotherapeutic agents). Braz J Med Biol Res, 2000; 33: 179-189.

6. Chan K., Some aspects of toxic contaminants in herbal medicines. *Chemosphere*, 2003; 52: 1361-1371.
7. David J., Newman, Gordon M., Cragg and Kenneth M., Snader. Natural Products as Sources of New Drugs over the Period 1981- 2002. *J. Nat. Prod.* 2003; 66: 1022-1037.
8. De Smet PAGM. Health risks of herbal remedies. *Drug Safety*, 1995; 13: 81-93.
9. Drew A., & Myers SP., Safety issues in herbal medicine: implications for the health professions. *Medical Journal of Australia*, 1997; 166: 538-541.
10. Drugs and Pharmaceuticals – Industry Highlights Incorporating Patent Information, CDRI, Lucknow, 1998; 21: 33–34.
11. Farnsworth N.R., Relative safety of herbal medicines. *HerbalGram*, 1993; 29: (Special Suppl): 36A-36H.
12. Gesler W.M., Therapeutic landscape: medicinal issue in light of the new cultural geography. *SocSci Med*, 1992; 34: 735-46.
13. Guyton, Hall. Textbook of medical physiology, Saunders, the Curtis center Philadelphia, Pennsylvania, 2000; 10: 798-801.
14. Harbone, J.B., In; Phytochemical Methods, A Guide To Modern Techniques Of Plant Analysis., 3rd edition. Springer (India) Pvt. Ltd., New Delhi, 2005; 5-16, 22.
15. Harsh Mohan. Textbook of Pathology, Jaypee brothers Medical Publishers (P) Ltd, New Delhi 2000; 4: 577-619.
16. Kamboj V.P., Herbal Medicine. *Current Science*, 2000; 78: 35-9.
17. Kenawy E.R., Worley S.D., broughton R; The chemistry and Applications of Antimicrobial polymers: A state-of-the-Art Review. *Biomacromolecules*, 2007; 8: 1359-1384.
18. Khandelwal, K.R., Practical Pharmacognosy, Techniques and Experiments, 11th edition. NiraliPrakashan, Pune., 2004; 149-156.
19. Kokate C.K., Practical pharmacognosy, 11th Edition, 1994; 108-109, 286.
20. Krishnaswamy N.R., Chemistry of Natural Products, A Laboratory Handbook, 1st edition. Universities Press (India) Pvt Ltd., Hyderabad, 2003; 5-20, 15, 26-31, 70-73.
21. Kritikar KR., and Basu BD., Indian Medicinal plants, 1984; II: 1561-1564.
22. Levy S.B., *Emerg Infect Dil*, 2001; 7(3): 512-515.
23. Lullmann H., Mohr K., Ziegler A., Bieger D. Color Atlas of Pharmacology. 2nd edn. Publication of Thieme Stuttgart. USA. 2000. IJPBA, Mar - Apr, 2011, Vol. 2, Issue, 2.

24. Manogaran E., National symposium on emerging trends in Indian medicinal plants, 2002; 10-12.
25. Miller L.G., Herbal Medicinals: selected clinical considerations focusing on known or potential drug-herb interactions. Arch Intern Med 1998; 158, 2200–11.
26. Mudur G., Mandatory rural practice proposed in India. BMJ 1995; 311, 1186.
27. Okwu D.E., and Josiah C., Evaluation of the chemical composition of two Nigerian medicinal plants. African Journal of Biotechnology 2006;5(4): 257-361.
28. Owais M, Sharad KS, Shehbaz and Saleemuddin M. Antibacterial efficacy of *Withaniasomnifera* (ashwagandha) an indigenous medicinal plant against experimental murine salmonellosis. Phytomedicine, 2005; 12: 229–235.
29. Patil A.S., Bhole S.R., Studies on life history and chemical control of semilooper on snake gourd. J Maharashtra AgriUni, 1993; 18(2): 229-231.
30. Rajgopal G., Ramkrishnan S., Practical Biochemistry for Medical Students, Orient Longman, 1983; 1-7.
31. Siana and Acalyphahispida. Phytother. Res, 14: 371-374.
32. Siow-Ping Tan., Eric Nyak-Yong Tan., *Phyllanthusacidus*: A review of its traditional uses, phytochemistry and pharmacological properties, J Ethnopharmacol, 2020; 253: 112610-112624.
33. Srivastava L.M., Das N., Sinha S., Essential of Practical Biochemistry, CBS Publication and Distributions, 2002; 40-50.
34. Surekha Yarnalkar., Practical Pharmacognosy, Nirali Prakashan, 1991; 38-47.