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Research Article

A Study of the Physico-Chemical and Phytochemical Parameters of Leaves of *Mallotus rhamnifolius*

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ABSTRACT
India being a rich and varied collection of medicinal plants since the Vedic age. The present study deals with the phytochemical screening of Mallotus rhamnifolius leaves of extraction belonging to family Euphorbiaceae. This study includes preparedness of different extracts by successive solvent extraction of varying polarity of Petroleum ether, chloroform, ethyl acetate, and Ethanol: water (95:5) extracts for detailed analysis. Phytochemical screening determinate by some chemical tests study was carried out for different solvent extracts. Phytochemical screening reflects existence of alkaloids, glycosides, saponins, phenolic compounds, tannins, phytosterols, carbohydrates, proteins, amino acids, flavanoids, quinones and terpenoids which may be responsible for their therapeutic effects. Different physicochemical parameters such as Loss on drying, Total ash, Acid insoluble ash and Water soluble ash were carried out as per WHO recommended physicochemical determinations and authentic phytochemical procedures. The results obtained in present study indicated Mallotus rhamnifolius leaves as a rich source of medicinally compounds and provides evidences that solvent extracts of Mallotus rhamnifolius contains medicinally important bioactive compounds and this justifies the use of plant species as medicine for treatment of various diseases.

Keywords: Mallotus rhamnifolius, Physicochemical parameters, Phytochemical screening.

INTRODUCTION

The esteem of all the drugs is based on phytochemical and pharmacological approaches which widen to drug discovery referred as natural product screening[1]. Any part of the plant may include active components like bark, leaves, flowers, roots, fruits, seeds, etc.[2]. The effects of the plant materials results when the secondary products such as phytochemicals get combined. In recent period concentration on plant research has increased all over the world. Plant have played a significant role in maintaining human health and improving the quality of human lifetime for thousands of years and have served humans as well as valuable components of medicines, seasonings, beverages, cosmetics and dyes[3]. Phytochemicals are the chemicals produces by plants, of these bioactive constituents of plants are steroids, terpenoids, carotenoids, flavanoids, alkaloids, tannins and glycosides. These compounds have various activities like antimicrobial or antibiotic, some have been reported to shows hemolytic and foaming activities[4], Anti-inflammatory[5], fugistatic[6] and molluscidal[7]. Thus the plants have played important role in drug development[8].

Mallotus rhamnifolius is commonly called as Buckthorn-Leaved Kamala. In Tamil- Marai-Yirdiyam, in Malayalam pee-tsjerou-ponnagam and Telugu- Konda-Kunkumu .Mallotus *rhamnifolius* is a small tree up to 5 m tall. Bark is greyish, warty. Branchlets are round, velvety. Leaves are simple, opposite to subopposite, decussate; stipules caducous; leaf-stalk 1-5 cm long, planoconvex in cross section, swollen at both ends, stellate hairy. Leaves are 10.5-23 x 5.3-13 cm, ovate, apex pointed to long-pointed, base rounded, entire or slightly sinuate, yellow-glandular beneath; trinerved at base; secondary nerves ca. 6 pairs. Flowers are unisexual, in axillary spikes. Capsule, 3-valved, minutely stellate hairy, seeds 3, brown.

Taxonomic Classification
Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Order: Euphorbiales
Family: Euphorbiaceae
Genus: *Mallotus*
Species: *Rhamnifolius*

MATERIALS AND METHOD

Collection of Plant Materials and Leaves

The leaves were collected from surroundings of the Ramakrishnapuram near to Kalakad is situated in the southern Western Ghats in Tirunelveli district, Tamil Nadu. This region is one of the plant diversity centers in India. The collected healthy leaves were washed with distilled water to remove the adhering unwanted particles. They were shade dried for 35 days and finally pulverized in to coarse powder. It was stored in a well closed container free from environmental climatic changes till usage.

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Preparation of the extract

Soxhlet extraction

About 80 gm of the dried leaves pulverization was extracted with hot solvents of increasing polarity such as petroleum ether, chloroform, ethyl acetate and ethanol : water (95:5) for 24 hours with each solvent, using by hot continuous extraction method[9] in Soxhlet apparatus at a temperature of 30˚C to 85˚C. Each time before extracting with next solvent, the powdered material was air dried and then subjected to further extraction. After the effective extraction, which they were filtered using Whatman filter paper No.1. Then the extracts was evaporated to solventlessness by using vacuum rotary evaporator to yields a soxhlet crude extracts to produce a sticky material, and further transferred into sterile bottles and refrigerated until use[10,11].

The percentage extractive yield was calculated by formula as mentioned below[12] and values show in Table No: 1

% Extractive yield (w/w) = weight of dried extract /weight of dried leave × 100

Physiochemical analysis

The physico-chemical parameters are mainly used in deciding the pureness and quality of the drugs. Ash values of a drug gives an idea of the earthy matters or inorganic composition or other impurities present along with the drug. The ash values of the powdered leaves revealed a high percentages of sulphated ash. Extractive value gives an idea about the chemical constituents present in the drug as well as useful in the judgment of exhausted or adulterated drugs[13]. The results suggests that the powdered leaves have high water soluble extractive value. The loss on drying reveals the percentages of moisture present in the leaves of Mallotus rhamnifolius are also studied and presented in Table No: 2.

Determination of Moisture (Loss on drying)

Measured about 1.5gm of the powdered leaves into a weighed flat and thin Porcelain dish. Dried it in the oven at 100^0C to 105^0C. Cool in desiccators and observed the loss in weight is usually recorded as moistures.

Ash values

The total ash, acid insoluble ash and water-soluble ash values were determined from air-dried samples[9,14]

Total ash value

Place about 2gm of the air-dried material, accurately weighed, in a previously ignited and tarred silica crucible. Spread the material in an even layer and ignite it by gradually increasing the heat to 500-600°C until it is white, indicating the absences of carbon. Allows the residue to cool in a desiccator for 30 minutes and then weigh without any time gap. Percentages of total ash was calculated with references to air-dried substance.

Total ash value of the sample[15] =100(Z-x)/y %

X= weight of empty dish

Y= weight of the drug taken

Z= weight of the dish + ash (after complete incineration)

Acid insoluble ash

To the crucible containing the total ash, add 25 mL of 2N Hydrochloric acid, close with a watch-glass and boil gently for 5 minutes. Wash the watch-glass with 5 mL of hot water and add this liquid to the crucible. Collect the insoluble matters on an ash less filter-paper and washing with hot water until the filtrate is neutral. Transfer the filter-paper containing the insoluble matters to the original crucible, dry on a hot plate and ignite to constant weight. Allows the residue to cool in desiccators for 30 minutes and then weigh without time gap. Calculate the content of acid-insoluble ash in mg per gm. of air-dried material.

Water soluble ash

To the crucible containing the total ash, add 25 mL of water and boil for 5 minutes. Collect the insoluble matters in a sintered-glass crucible. Wash with hot water and ignite in a crucible for 15 minutes at a temperature not exceeding 450 °C. Decreases the weight of this residue in mg from the weight of total ash. Calculate the content of water-soluble ash in mg per gm. of air-dried material. Percentage of water-soluble ash was calculated with reference to air-dried substance[16].

Phytochemical Screening

The qualitative preliminary phytochemical tests were performed for testing different chemical groups present in extracts. The primary metabolites like proteins, carbohydrates and fixed oils and fats, were analyzed for their presences as per the standard procedures[17, 18,19,20,21]. Similarly, the secondary metabolites like, alkaloids, flavonoids, saponins, phenolic,

tannins, terpeniods and glycosides were also assessed in the leaves extracts of Mallotus rhamnifolius and results showed in table no: 3.

Test for alkaloids

An amount of 0.5g of concentrated extracts was taken with 5 mL of the 1% aqueous HCl acid the mixture was heated gently for 20 min cooled and filter, the filtrate was used for following tests.

Mayer's Test

Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow colored precipitate indicates the presences of alkaloids.

Dragen droff's test

A few drops of Dragen dorff's reagent were used to react with 1 mL of the filtrate, turbidness or precipitation with this reagent was taken as evidence for the presences of alkaloids[22].

Hager's test

1mL of the extract was treated with Hager's reagent, presences of alkaloids confirmed by the yellow colored precipitate.

Wagner test

1mL of the extracts was treated with Wagner's reagent; formation of brown reddish precipitate indicates presences of alkaloids.

Test for Sugars and Carbohydrates

Barfoed's test

Take the equal volumes of Barfoed's reagent and the extracts solution. Heated for 1-2 min in a boiling water bath and cooled. Red color was observed[23].

Keller-Killani Test

Plant extracts treated with 2 mL glacial acetic acid containing a drop of $FeCl_3$. A brown color ring indicates

Table 1: Physical characteristics and % yield of Mallotus rhamnifolius.

Solvent	Color of extract	Sense of touch	Amount of extract (gm)	% yield
Petroleum ether	Brownish dark green	Sticky	4.67	5.83%
Chloroform	Brownish dark green	Sticky	10.21	12.76%
Ethyl acetate	Reddish Brown	Sticky	14.93	18.66%
Ethanol: water (95:5)	Reddish Brown	Sticky	28.45	35.56%

Table 2: Physicochemical characterization of leaf of Mallotus rhamnifolius.

Physicochemical characters	Average values % w/w Leaves
Loss on drying	5.7
Total ash	6.07
Acid insoluble ash	0.72
Water soluble ash	1.31

the presences of positive test.

Legal's Test

To the extracts 1mL of pyridine and few drops of freshly prepared sodium nitroprusside solution were added, formation of pink to red color indicates presences of glycosides.

$FeCl_3$ Test

Added 2 mL of glacial acetic acid containing 1 drop of ferric chloride solution and 1 mL of conc. H_2SO_4. Formation of a brown ring indicates the presences of cardiac glycosides[24].

Fehling test

Mixed of 1 mL of Fehling's A and Fehling's B solutions, boiled for one minute. Add equal amount of test solution. Heated in a boiling water bath for 5-10 min. First a yellow then a brick red ppt. was observed[23].

Benedict's test

Filtrate were treated with Benedict's reagent and heated gently, orange red precipitate indicates the presences of reducing sugar.

Test for steroids

H_2SO_4 acid test

1 mL extracts was dissolved in 10 mL of chloroform and equal amount of concentrated H2SO4 acid was added from the wall of test tube .The upper layer turns red and H2SO4 layer showed yellow with green fluorescence. This indicates the presences of steroid.

Libermann Burchard's test

Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. Appearance of brown ring at the junction indicates the presences of Steroid.

Test for Tannin

$FeCl_3$ test

4ml extracts was treated with 4 mL FeCl3 appearance of green color indicates that of tannin23.

Lead acetate test

To 1-2 mL of extracts, basic lead acetate was added separately. Appearance of bulky red precipitate indicates the presences of tannins.

Gelatin Test

To the extract, 1% gelatin solution containing sodium chloride was added. Appearance of white precipitate indicates the presences of tannin24.

Test for proteins and amino acids

Million test

3 mL test solutions were mixed with 5 mL Million's reagent, White precipitate was formed which on heating turned to brick red. It may indicates the presences of amino acids.

Biuret test

Added 4% of NaOH and few drops of 1% $CuSO_4$ solution to 3 mL of the extracts. Appearance of violet or pink color indicates the presences of proteins[23].

Xanthoproteic test

Extracts was treated with few drops of concentrated HNO_3

appearance of yellow indicates the presences of proteins.

Ninhydrin test

To the 2 mL extracts 2 mL on ninhydrin reagent was added and boil for few minutes, appearance of blue color indicates the presences of amino acid.

Test for terpenes

H_2SO_4 test

To 5 mL of the extracts add 2 mL of chloroform and 3 mL of conc.H_2SO_4, appearance of a reddish brown ring confirms the presences of terpenes[25].

Test for flavonoids

Shinoda test (Magnesium Hydrochloride reduction test)

5mL extracts added with 95% ethanol, then treated with 0.5g magnesium turnings and few drops of conc. HCl pink color, indicates the presences of flavonoids.

Alkaline reagent test

Extract was treated with 10 % NaOH solution, formation of intense yellow color indicates presences of flavonoids.

NH_4OH test

3 mL of extracts were 10 % NH_4OH solution development of yellow fluorescence indicates positive test.

Conc. HCl test

A few drops of concentrated hydrochloric acid were added to a small amount of the extracts of the plant material. Immediate development of a red color was taken as an indicates of the presences of flavonoids[26].

Zn test

2 mL extracts were treated with Zn dust and conc. HCl development of red color indicates presences of Flavonoids.

Test for Anthocyanin

HCl test

2 mL of aqueous extract is added to 2 mL of 2N HCl and NH_3, the formation of pink red turns blue violet indicates presences of Anthocyanin.

Test for Quinones

Table 3: Phytochemical screening of various extracts of Mallotus rhamnifolius.

S.No	Constituents	Test	Pet. Ether	Chloro Form	Ethyl acetate	Ethanol: Water
1		Mayer's reagent	-	-	+	+
2	Alkaloids	Dragendorff's reagent	-	-	+	+
3		Hager's reagent	-	-	+	+
4		Wagner's reagent	-	-	+	+
5		Barfoed's test	-	+	+	+
6		Keller-Killiani test	-	-	+	+
7	Sugars &	Legal's test	-	-	+	+
8	Carbohydrates	Ferric chloride test	-	-	+	+
9		Fehling's test	-	+	+	+
10		Benedict's test	-	+	+	+
11		H_2SO_4 acid test	+	+	-	-
	Steriod	Libermann				
12		Burchard's test	+	+	-	-
13		Ferric chloride test	-	+	+	-
14	Tannins	Lead acetate test	-	+	+	-
15		Gelatin solution	-	+	+	-
16		Millon's test	-	-	+	+
17	Protein &	Biuret test	-	-	+	+
18	Amino acid	Xanthoprotein test	-	-	+	+
19		Ninhydrin test	-	-	+	+
20	Terpenoids	H_2SO_4 test	-	-	+	+
21		Shinoda test	+	+	+	+
22		Alkaline reagent test	+	+	+	+
23	Flavonoids	NH_4OH test	+	+	+	+
24		Conc. HCl test	+	+	+	+
25		Zn test	+	+	+	+
26	Anthocyanins	HCl test	-	-	-	+
27	Quinone	Alc. KOH test	-	-	-	+
28	Saponin	Frothing test	-	-	-	+
29	Phenol	Ferric chloride test	-	-	+	+
30	Coumarin	NaOH test	-	-	-	+
31	Anthraquinone	H_2SO_4 test	-	-	+	+

Alc. KOH test

The extracts were treated separately with Alc. KOH solution. Formation of red color indicates the presences of Quinones.

Test for saponins

Frothing test

Exactly 0.5 g of the extracts was dissolved in distilled water in a test tube. Frothing which persisted on warming was taken as preliminary conformation for saponins[22].

Test for Phenol

Ferric Chloride test

Test extracts were treated with 4 drops of alcoholic $FeCl_3$ solution. Appearance of bluish black color indicates the presences of Phenol[23].

Test for Coumarin

NaOH test

3 mL of 10% NaOH was added to 2 mL of aqueous extracts appearance of yellow color indicates coumarins.

Test for Anthraquinone

H_2SO_4 test

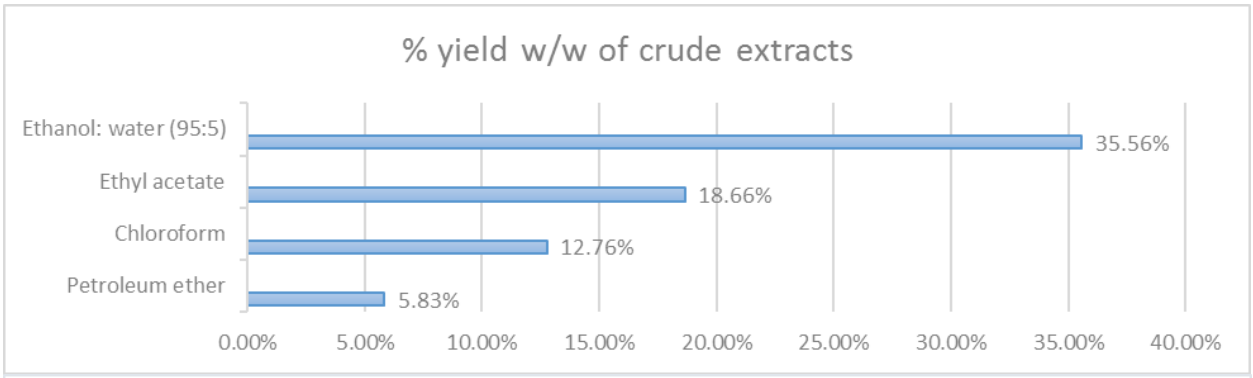
5mL of Extract was hydrolyzed with dilute H_2SO_4 then add 1mL of benzene and 1mL of NH_3 solution appearance of rose pink coloration suggest presences of anthraquinone.

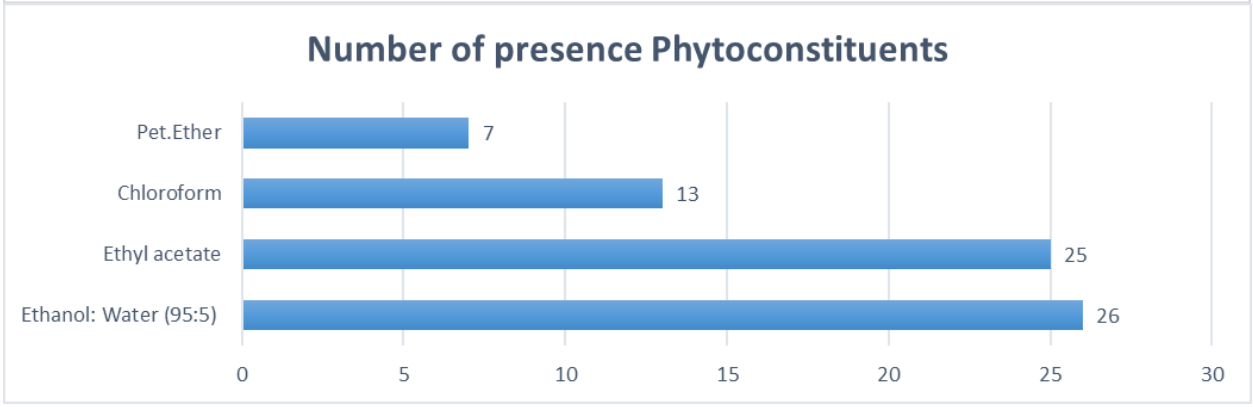
RESULTS AND DISCUSSION

All the results generated from the present investigating are represented in the respective tables. The powdered leaves of Mallotus rhamnifolius was subjected to preliminary physio chemical and phytochemical analyses which were find out to be very promising.

The extracts (petroleum ether, chloroform, Ethyl acetate and Ethanol extracts) of Mallotus rhamnifolius were prepared by the hot continuous extraction method. In the present work the percentage yield w/w of crude extracts was also analyzed wherein the highest yield (35.56%) obtained with Ethanol: Water (95:5) and least (5.83%) with petroleum ether media. The color of the extracts was find out to be Brownish dark in petroleum ether extract, Brownish dark in chloroform, Reddish brown in Ethyl acetate extracts and Ethanol: water extract.

Deterioration duration of the plant material depends upon the quantity of water present in plant material. If the water content is high, the plant can be easily deteriorated due to impureness by fungal colonies. The loss on drying





at 100^0C to 105^0C in leaf was find out to be 5.7 %. The total ash value of plant material indicated the quantity of minerals and earthy materials attached to the plant material. The analytical results showed that total ash value content was 6.07 %. Similarly, negligible quantity of acid insoluble siliceous matter present in the plant was 0.72% and 1.31% of Water soluble ash was observed.

Phytochemical screening showed that maximum presences of phytoconstituents in Ethylacetate and Ethanol: Water (95:5) extracts. In the present research Ethanol : water extract shows the presences of 26 phytoconstituents tests, Ethylacetate extracts contains 25 phytoconstituents tests, whereas Chloroform extracts contain 13 phytoconstituents tests and Petroleum ether extract contain 7 phytoconstituents tests from the Mallotus rhamnifolius. From above studies Ethanol: water extracts contains more number of phytochemicals whereas Petroleum ether extracts shows less number of phytochemicals.

CONCLUSION

In conclusion, the present research revealed the potential of phytoconstituents present in the leaves of Mallotus rhamnifolius in terms of phytochemical parameters. Moreover, further experiments are needed to a quantify the chemical compounds and find out bioactivities of the leaves of Mallotus rhamnifolius.

REFERRENCE

1. Foye WO, Lemke TL, Williams DA, Foye's Principles of Medicinal Chemistry, 6th Ed Lippincott Williams and Wilkins.Philadelphia, 44-45, (2008).
2. Gordon MC, David JN. Natural product drug discovery in the next millennium. Pharm Biol. 39 Suppl 1:8-17,(2001).
3. Makhija I.K., Sharma I.P., Khamar D.;Phytochemistry and Pharmacological properties of Ficus religiosa: an overview; Scholars Research Library; Annals of Biological Research, 1 (4) : 171-180,(2010).
4. Feroz, M., R. Ahmad and S.T.A.K. Sindhu and A.M. Shahbaz, 1993. Antifungal activities of saponins from indigenous plant roots. Pak. Vet. J., 13: 4-4, (1993).
5. Takagi, K., Hee P.E. and Histoshi K., Antiinflammatory activities of hedergenin and crude saponin from Sacchindus mukarassi. Chem. Bull. 28(4): 1183 (Eng.) Chem. Abst. 93(21): 372384), (1980).
6. Zehavi, U.M.L. and R. Segel,. Fungistatic activity of saponin A from Styrex officinalis L. on plant pathogens. J. Phytopathol., 116: 338-343. (1986).
7. Sati, O.P., Chandra C.D. and Usha R Molluscidal saponins of xeromphis saponins. Planta Medica, 5: 981-983,(1987).
8. Edeoga HO, Okwa DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. African Journal of Biotechnology, 4(7): 685-688, (2005).
9. Tasneef Ahmad, Swatantra Bahadur Singh, Shivshankar Pandey, Phytochemical Screening and Physicochemical Parameters of Crude Drugs: A Brief Review International Journal of Pharma Research & Review, Dec 2013; 2(12):53-60.
10. Subhashini R, Mahadeva-Rao US, Sumathi P, Gunalan G, A comparative Phytochemical analysis of cocoa and green tea, Indian J Sci., Technol; 3 (2):188-192, (2010).

11. Sofowara A, Medicinal Plants and Traditional Medicine in Africa. Spectrum Books Ltd., Ibadan, Nigeria: 1993. (289-300).
12. Ashok Kumar1, K.K. Jha1, Dinesh Kumar, Abhirav Agrawal and Akhil Gupta www.thepharmajournal.com THE Pharma Innovation Vol. 1 No. 5 2012 Page71.
13. R. Vadivu, N.Jayshree, C.Kasthuri, K.Rubhini and G.Rukmankathan,Pharmacognostical standardization of leaves of Ixora coccinea, linn. J. Pharm. Sci. & Res. Vol.2 (3), 2010, 164-170.
14. WHO Library Cataloguing-in-Publication, Data Quality control methods for herbal materials.Updated edition of Quality control methods for medicinal plant materials, 1998.
15. Khandelwal k.r., practical pharmacognosy 12th edition, Nirali Prakashan, 2004, 159.
16. B Abhishek, Saini Avinash, Evaluation of Physiochemical Screening and Standardization on the Root of Rotula aquatica Lour. Vol. 1, Issue 1, 2013, 63-68.
17. Trease G.E., and Evan W.C., Pharmacognosy, Ed 12, English language Book society, Balliere Tindall, 309-315 and 706-708 (1983).
18. Kokate C.K, Purohit A. P. and Ghokhale S.B. "Pharmacognosy" 17th edition , Nirali Prakashan, 2009 pp. 99, 231, 185, 271, 445, Pune.
19. Hegde Karunkar and Joshi Arun B, Scholars Research Library Der Pharmacia lettre 2(3):255 (2010).
20. Harborne JB, Methods of extraction and isolation. In Phytochemical Methods. Chapman & Hall,London, 1998, 60-66.
21. Brain KR and Turner TD. The Practical Evaluation of Phytopharmaceuticals. Wright- Scientechnica. Bristol, 1975, 36-45.
22. Adegoke, A.A., Iberi, P.A., Akinpelu, D.A., Aiyegoro, O.A., Mboto,C.I., 2010. Studies on phytochemical screening and antimicrobial potentials of Phyllanthus amarus against multiple antibiotic resistant bacteria. Int. J. Appl. Res. Nat. Prod. 3 (3), 6–12.
23. Boxi, M., Rajesh, Y., Raja, Kumar V., Praveen, B., Mangamma, K., 2010. Extraction, phytochemical screening and in-vitro evaluation of anti-oxidant properties of Commicarpus chinesis (aqueous leaf extract). Int. J. Pharm. Biosci. 1, 537–547.
24. Prashant Tiwari, et al: Phytochemical screening and Extraction: A Review Internationale Pharmaceuticasiencia Jan-March 2011 Vol. 1, Issue 1.
25. Obianime, A.W., Uche, F.I., 2008. The phytochemical screening and effects of methanolic extract of Phyllanthus amarus leaf on the biochemical parameters of male guinea pigs. J. Appl. Sci. Environ.Manage. 12 (4), 73–77.
26. Shahid-Ud-Duaula, A.F.M., Anwarul, Basher M., 2009. Phytochemical screening, plant growth inhibition, and antimicrobial activity studies of Xylocarpus granatum Malaysian. J. Pharm. Sci. 7 (1), 9–21.