Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2017; 9(5); 733-738

ISSN: 0975-4873

Research Article

Pharmacognostic Evaluation, Phytochemical Screening and Antimicrobial Activity of Stem Bark of *Ficus Krishnae*

Amarvani P Kanjikar, Ramesh L Londonkar*

Department of Biotechnology, Biopharmaceutical and Nanobiotechnology, Gulbarga University, Kalaburagi. India.

Received: 18th January, 17; Revised 30th April, 17, Accepted: 15th May, 17; Available Online:25th May, 2017

ABSTRACT

Ficus krishnae is a perennial plant, used in number of folklore medicine, to treat ulcer, vomiting, fever, inflammation, diabetes, dysentery, leprosy and cancer etc. The present research deals with the study of pharmacognostic, phytochemical and antimicrobial activity of stem bark of *Ficus krishnae.* The pharmacognostic parameters for the study were physicochemical, fluorescence analysis and preliminary phytochemical screening along with mineral analysis. In physicochemical evaluation the ash value and extract value were studied. The antimicrobial activity of the stem bark extract against pathogenic strains was evaluated based on the inhibition zone using well diffusion assay; minimum inhibition concentration (MIC) is studied using micro dilution method. The powder of *Ficus krishnae* was successively extracted with petroleum ether, chloroform, methanol and aqueous by hot soxhlet extraction. The preliminary screening was carried out for different extract. Fluorescence analysis has showed the normal ranges of fluorescence colour for the crude powder. The preliminary phytochemical screening resat also indicated the presence of principle active compounds of *Ficus krishnae* which include phenols, flavonoids, saponin, alkaloids, glycosides, oils, fats, steroids, proteins and carbohydrates. The inorganic element analysis study indicated the presence of calcium, potassium, magnesium, manganese, zinc, molybdenum, vanadium, titanium and cadmium. In the present study we have tested antimicrobial activity of Petroleum ether, chloroform, methanol and Aqueous extractions of stem bark of *Ficus krishnae* plant against six bacteria (*E.coli, Staphylococcus aureus, ENT, Enterococcus fecalis, salmonella typhi*,) and one fungi (*Aspergillus niger*). These studies gives information of physicochemical character and preliminary property of stem bark of *Ficus krisnae* for the identification of crude drug and it promote the search for isolation of medicinally valuable active compounds.

Keywords: *Ficus krishnae*, pharmacognostic, phytochemical screening, mineral analysis, antimicrobial activity.

INTRODUCTION

The medicinal plants have been used to cure different health associated problems in many countries of the world since time of prehistoric[1]. In recent years most of the synthetic drugs have raised health relevant problems because of their side issue[2] and developed multiple resistivity by pathogenic microorganisms. In addition to this problem, antibiotics are sometimes associated with causing adverse effects on the host including hypersensitivity, immune-suppression and allergic reactions. Due to this, plant based medicines are more accepting around the world. *Ficus* is one of the bulkiest genuses with 800 species occurring all over the world, graded twenty-first in angiosperms[3,4]. One hundred and fifteen species are distributed throughout the India and approximately 43 species are found in Meghalaya alone[5].

Ficus krishnae is also known as Makkhann Katori in Hindi and Krishna fig or Krishna's butter cup in English. It is mainly found in India, tropical Africa and Sri Lanka[6]. The plant has 10 m in height, fast growing tree with spreading branches and aerial roots. Leaves are simple, dorsiventral with acute apex, entire margin and reticulate venation. Dorsal surface of the leaf is dark green while ventral surface is light green in colour. The unique character of the tree is that the pocket like fold at the base of leaf. It has been used in number of folklore medicines. Various parts of the plant are used to treat ulcers, vomiting, fever, inflammations and leprosy. The plant is also used as aphrodisiac, as a tonic, in piles and gonorrhoea. Stem bark and leaves are useful in treatment of diabetes. The aerial roots are styptic; useful in syphilis, biliousness, dysentery and inflammation of liver[7,8]. This property indicates the medicinal importance of this species. Based on the above information on the plant has been selected for present investigation.

MATERIALS AND METHODS

Collection of Plant Material and Extraction

Stem bark of *Ficus krishnae* was collected by Dev Dev vana botanical garden, Bidar, Karnataka. The plant is duly identified by Department of Botany, Gulbarga University Kalaburagi, Karnataka, India.

The stem bark was allowed to dry in shade for two to four weeks. After drying, the bark was grinded into finely powder and stored in airtight container. The air dried bark powder (100 g) was successively extracted by soxhlet extraction with solvents of increasing polarity i.e., petroleum ether, chloroform, methanol and aqueous. The

*Author for Correspondence: londonkarramesh53@gmail.com

Table 1: Physiochemical parameters.

Sl no	Parameter	Percentage of ash
01	Total ash	5.62%
02	Acid insoluble ash	1.41%
03	Water soluble ash	4.62%

extracts were dried and stored in a sterile container for further use.

Pharmacognostic Studies

The ash values and extractive value were determined according to the standard methods prescribed in Indian Pharmacopeia[9] and also as per the WHO guidelines on quality control methods for medicinal plants materials. Fluorescence analyses were carried out according to the method of Chase and Pratt 1958[10] and Kokoski 1995[11].

Phytochemical Screening

Petroleum ether, chloroform, methanol and aqueous extracts were subjected to comparative phytochemical analysis for the presence of various secondary phytoconstituents using standard procedure described by kokatte[12] and Horborne[13]. The extract residues of the plant were subjected to phytochemical screening to detect the presence of various active phytocompounds like phenols, tannins, flavonoids, saponins, alkaloids, primary metabolites like carbohydrates, proteins and lipids.

Mineral analysis

The mineral like Calcium, Copper, Iron, Magnesium, Potassium, Manganese, Vanadium, Titanium, Molybdenum and Zinc in stem bark of plant were determined using the standard methods given by NIN[14]. All the determinations were done in triplicates.

Antibacterial assay

Test microorganisms

The test organisms used in this experiment includes four Gram positive bacteria strains, two Gram negative bacterial strains namely *Enterobacter aerogenes* (MTCC 111), *Escherichia coli* (MTCC 45*), Shigella dysenteriae*(clinical isolate), *Salmonella typhimurium*(MTCC 98),*Staphylococcus aureus* (ATCC 29122) and *Enterococcus faecalis* (ATCC 29212) respectively. Fungal strain is *Aspergillus niger*(MTCC 282) all the samples were procured from IMTECH, Chandigarh, India.

Culture media

Nutrient broth (NB), Nutrient media, Sabouraud Maltose Agar and Potato dextrose agar (PDA) media were procured by Hi Media Laboratories Ltd., India.

Well Diffusion Method

In vitro antibacterial activities of all different extracts of stem bark of *Ficus krishnae* were determined by standard agar well diffusion assay. Nutrient agar plates were seeded with 16 hr old culture of the isolates. Different extracts were dissolved in 10% Tween 80 and 20% DMSO in deionized water and made the final concentration of 100 mg/ml; from this 100 µl of different extracts were added into the sterile 5 mm diameter well. 10% Tween 80 and sterilized distilled water were used as negative controls, while cefotaxime (Gram –ve bacterial), Vancomycin (Gram +ve bacteria) antibiotic disc (30 mcg, Hi-Media) was used as positive control. A standardized culture of test organisms were streaked on the solidified medium and incubated for 24 h at 37º C. After incubation the diameter of the zone of inhibition formed around the well was measured using standard (Hi-Media) scale. The experiment done in triplicate and the average values were calculated for antibacterial activity.

Minimum inhibitory concentration

The minimum inhibitory concentrations (MIC) of the plant extracts were determined using the broth dilution method according to Clinical and Laboratory Standard Institute (CLSI) methodology[15]. In this method, 1ml of the extract solution at the concentration of 50mgml^{-1} was added to 1ml of nutrient broth and subsequently transferred to make solutions of varying concentrations (20, 40, 60, 80 and 100 mgml^{-1}) in different test tubes. Then 1ml of bacterial and fungal suspensions and 0.1ml of plant extracts were added to each test tube and incubated at 37˚C, 24h for bacteria and 25˚C, 48h for fungi. The test tube with the concentration of plant extract at which no detectable growth was observed is considered as the MIC.

Table 2: Fluorescence analysis of powder.

Sl no	Treatment	Ordinary light	UV light
01	Powder	White colour	White colour
02	Powder + distilled water	Light yellow	Light blue
03	Powder + petroleum ether	Light yellow	Light blue
04	Powder + chloroform	Light brown	Light pink
05	Powder + methanol	Light yellow	Light blue
06	Powder + concHcl	Light brown	Light pink
07	Powder + concHNO3	Yellow brown	Light pink
08	Powder + concH2SO4	Light brown	Light blue
09	Powder + picric acid	Yellow	Greenish yellow
10	Powder + ammonia	Brown	Light blue
11	Powder + 1N NaOH (aqueous)	Brown	Light blue
12	Powder + 1N NaOH (alcohol)	Transparent	Light blue
13	Powder + acetic acid	Yellow	Light blue
14	Powder + Ferric chloride	Brown	Pink
15	Powder + 5% KOH	colourless	colourless
16	Powder + ethyl acetate	colourless	Light purple

Table 3: Qualitative analysis of primary and secondary metabolites of stem bark.

Sl no	Test	Petroleum ether	chloroform	Methanol	Aqueous
01	Carbohydrate	+	+	+	+
02	Protein	+	+	+	+
03	Oil and fat	+	+	+	+
04	Phenol	-	-	+	+
05	Flavonoid	-	-	+	+
06	Steroids	+	+	+	-
07	Saponin	-	-	+	-
08	Alkaloid	-	+	+	+
09	Tannin	-	-	+	+
10	Glycoside	+	+	+	-

Table 4: Shows Mineral Analysis.

Elements	Concentration ppm
Calcium (Ca)	1.5169
Potassium (K)	0.2199
Magnesium (Mg)	1.0934
Manganese (Mn)	0.0291
Zinc (Zn)	0.0325
Molybdenum (Mo)	0.1712
Vanadium (V)	0.5775
Titanium (Ti)	1.7649
Cadmium (Cd)	0.0196

Assay for antifungal activity

The in vitro antifungal activity of the plant extracts was evaluated using modified disk diffusion method. Potato Dextrose Agar was dispensed into petri dishes and allowed to solidify. Spores were recovered by gentle swabbing the surface of the culture plates using a sterile cotton swab; later the swab was dipped in 3 ml sterile saline containing 0.1% Tween 80 to suspend the spores. Agar wells of 5 mm diameter were made with the help of a sterilized stainless steel cork borer. Aseptic conditions were maintained during the loading of test extracts marked agar wells using micropipette and water was considered as negative control and Amphotericin antibiotic as positive control. Plates were incubated at 25 ◦C for 48h and 72 h. The zone of inhibition was measured in mm.

RESULTS

Physicochemical studies

The physical appearance, colour and percentage of petroleum ether extract of *Ficus krishnae* L was sticky yellow with 0.36% yield. whereas chloroform, methanol and aqueous extracts are appeared as semi solid brown with 0.45%, 02% and 03% respectively.

The total ash, acid soluble ash, water soluble ash and moisture content of stem bark of *Ficus krishnae* was done and the result were recorded in Table-1.

Fluorescent studies

Fluorescence analysis of stem bark of *Ficus krishnae* powder with different chemical reagents were detected under ordinary and UV light to be detect the fluorescent compound and results are tabulated in Table-2. When stem bark powder was treated with distilled water, petroleum ether, methanol and conc H_2SO_4 it shows yellow colour under ordinary light, whereas blue colour under UV light. But in chloroform, con Hcl, concHNO_3 and $FeCl_3$ exhibit the brown colour under ordinary light, whereas pink colour under UV light.

Phytochemical screening

The phytochemical test for primary and secondary metabolite of stem bark extract releaved the presence of protein, carbohydrates, glycosides, saponins, steroids, tannins, flavonoids, alkaloids, oils, fats, glycocides and phenol shown in the Table-3. The petroleum ether and chloroform extracts have shown the presence of carbohydrates, proteins, oils, fats, steroids, alkaloids and glycosides. Whereas methanol extract has shown the positive test for carbohydrates, protein, oils, fats, phenols, flavonoids, steroids, saponins, alkaloids and glycosides. The aqueous extract has showed the presence of carbohydrate, proteins, oils, fats, phenols, alkaloids and tannins.

Mineral analysis

The AAS elemental analysis of the plant stem bark was presented in the Table-5. The values of calcium, potassium, magnesium, manganese, zinc, molybdenum, vanadium, titanium and cadmium were reported in parts per million (ppm).

Antimicrobial assay

The results of agar well diffusion and minimum inhibitory concentration are shown in Table 6 and Table 5 respectively, indicates the antibacterial activity against pathogenic organisms exhibited by all three extracts. The zones of inhibition were shown in the Figur-1 Comparatively methanolic extract has shown the higher activity followed by petroleum extract, aqueous and chloroform extracts.

DISCUSSION

The improvement in the quality control and standardization of herbal drugs has led to the development of effective quality medicines from plants. The present investigation hits the pharmacognostic evaluation of crude drug for judging acceptability or rejection of crude drugs in the medicines. Important parameter for evaluation of crude drug by physical constant evaluation is to detect the adulteration or improper handling of drugs[17]. Evaluation of crude drugs is the ash value, acid insoluble ash value and water soluble ash value determination. The total ash is particularly important in the evaluation of purity of drugs, i.e., the presence or absence of foreign organic matter such as metallic salts and/or silica[18]. The ash value of Krishnae

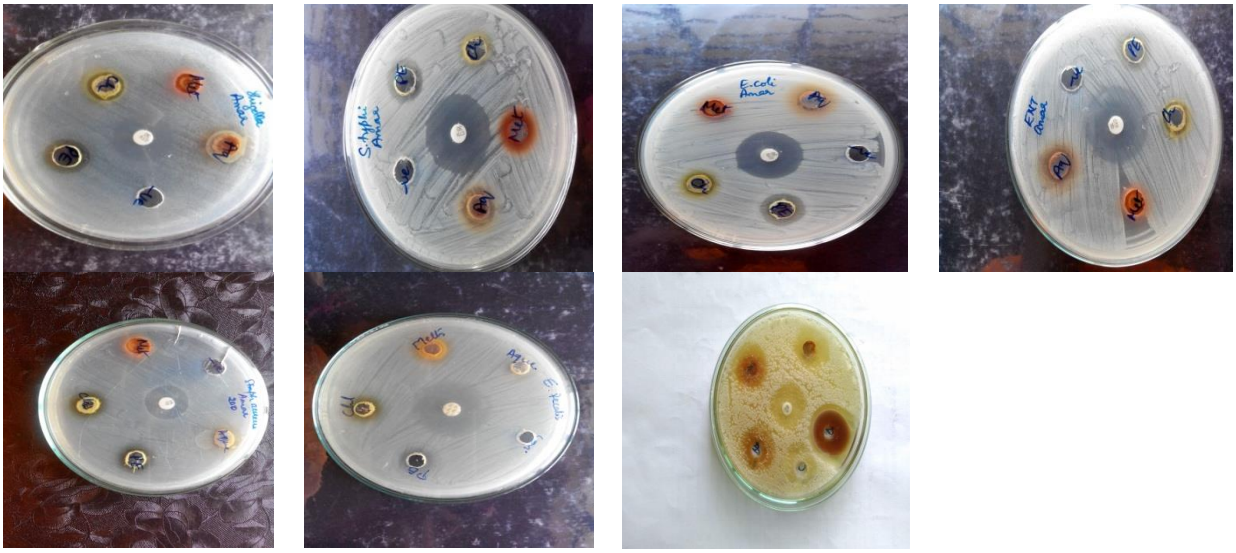

Figure 1: Zone of inhibition of micro organism of defferent extracts of *Ficus krishnae* (shegella, S typhi, *E –coli, ENT, S areous, E-feccalis and Aspergillus niger).*

Table 5: MIC of bark extracts on bacterial and fungal strains of *Ficus krishnae* stem extract (in µl).

Sl no	Test Organism	Strains	Petroleum ether	Chloroform	Methanol	Aqueous
01	*Enterobacter aerogenes*	MTCC 111	100	100	80	100
02	*Escherichia coli*	MTCC 45	100	100	100	100
03	*Shigella dysenteriae*	Clinical isolate	100	100	80	100
04	*Salmonella typhimuriu*	MTCC 98	100	100	80	100
05	*Staphylococcus aureus*	ATCC 29122	100	100	100	100
06	*Enterococcus faecalis*	ATCC 29212	100	100	100	100
07	*Aspergillus niger*	MTCC 282	100	100	100	100

fig stem bark powder is 5.62%. This ash value is indicative of the impurities present in the drug; this value is also one of the diagnostic parameters of the drug. In the present study, the stem barks powder has more acid insoluble ash value than water soluble ash value. In herbal drugs, variable limit of water are present. The physicochemical parameters such as percentage of ash, extractive value and foreign matter content are determined in triplicate and the results are in line with the findings of Kadam PV et al., (2012)[19].

Fluorescence is the phenomenon to exhibit various colour due to chemical constituents present in the plant material. The fluorescence colour is specific for each phytocompound. The non-fluorescent compound may fluorescent if it is mixed with impurities that are fluorescent. The fluorescent method is adequately sensitive and enables the precise and accurate determination of pharmaceutical samples[20]. In the present study, the powdered stem bark of *Ficus krishnae* emitted medium range of color under daylight and under UV light after treatment with various acids, alkalis & other reagents. Florescence analysis of powders gives a clue if powder is in adulteration thus can be used as a diagnostic tool for testing the adulteration. This procedure is pre-requisite before going for detailed photo chemical investigation. The phytochemical screening of Krishnae fig stem bark was undertaken for the identification of different chemical constituents present in different extracts. The petroleum ether, chloroform, methanol and aqueous extracts of stem bark obtained by successive soxhlet extraction has showed the presence of phenols, flavonoids, sterols, saponins, protein and carbohydrates.

The human beings require a number of complex organic/inorganic compounds in diet to meet the need for their activities. The important constituents of diet are carbohydrates, fats, proteins, vitamins, minerals and water[21]. Inorganic elements play an important role in physiological process involved in human health. Potassium is diuretic and it takes part in ionic balance of the human body and maintains tissue excitability. Potassium ions are transmit electrical impulse in the nerve cells and maintain the fluid balance of the body.

Calcium imparts strength and rigidity to bones and teeth. Calcium ions are also needed in neuromuscular transmission, normal excitability of heart, in clotting of blood and promoting muscular contraction. Magnesium is the fourth most abundant cat ion in the body. In muscles and other tissues, a magnesium ion activates many enzymes involved in carbohydrate metabolism, synthesis of nucleic acids (DNA and RNA) and protein synthesis. Tracer amount of magnesium causes irritability of the nervous system, peripheral vasodilation and cardiac

Table 6: Zone of inhibition (mm) of micro organisms by well diffusion method of *F.krishnae.*

l no	Test Organism	Strains	Petroleum Ether	Chloroform	Methanol	Aqueous	Control	Positive control
01	*Enterobacter aerogenes*	MTCC 111	1.2	1.3	1.5	0.6	00	2.7
02	*Escherichia coli*	MTCC 45	1.5	1.2	1.5	1.4	00	2.3
03	*Shigella dysenteriae*	Clinical isolate	1.4	1.3	1.5	0.8	00	2.0
04	*Salmonella typhimuriu*	MTCC 98	1.4	1.2	1.6	1.3	00	2.6
05	*Staphylococcus aureus*	ATCC 29122	1.2	1.4	1.3	0.9	00	1.8
06	*Enterococcus faecalis*	ATCC 29212	1.3	1.3	1.2	1.5	00	1.9
07	*Aspergillus niger*	MTCC 282	1.1	1.4	1.7	1.0	00	1.6

PE-Petroleum ether, CH-Chloroform, ME-Methanol and AE-Aqueous extract
C-control, +ve control is cefotaxime (Gram –ve bacterial), Vancomycin (Gram +ve bacteria) and Amphotericin (Fungi)

arrhythmias[22]. Manganese is essential for haemoglobin formation but excess is harmful. Zn is an essential component of a number of enzymes present in animal tissue including alcohol dehydrogenase, carbonicanhydrase, procarboxypeptidase and is for normal growth, reproduction, tissue repair and wound healing. Zinc deficiency causes growth retardation and skin lesions[23]. Vanadium supplements are used as medicine, used for treating diabetes, low blood sugar, high cholesterol, heart disease, tuberculosis, syphilis anemia, edema and for preventing cancer. Titanium is biocompatible (non-toxic), has many medical uses, including surgical implements and implants, such as hip balls and sockets and dental implants. Molybdenum has an important role in normal body functions, low amounts of molybdenum in the body, risk of esophageal cancer. Every constituent plays an important role and deficiency of any one of mineral constituent may lead to abnormal developments in the body.

According to above antimicrobial activity results, at a high concentration of 100µg/mL, it was observed that the petroleum ether, chloroform, methanol and aqueous extracts of all the tested isolates exhibited good antibacterial and antifungal activities. At a low concentration of 12.5µg/mL, the aqueous extracts could not inhibit the growth of the micro organisms but the methanol extracts was still very active with inhibition. All the extracts were longer inhibition to fungal growth at 100µg/mL on Aspergillus niger at this concentration. At lower concentration of all extract were no longer susceptible to fungal growth at 12.5µg/mL.

The inhibitory activity of *Ficus krishnae* extracts against pathogens confirmed by the potential use of the plant in the treatment of bacterial diseases, but moderate effect was observed against fungal disease. Presence of different chemical compounds in the extract impart significant amount of biological activities of *F. Krishnae* thus proving its medicinal value.

CONCLUSION

Ficus krishnae is one of the most ancient folk medicine. The pharmacognostic investigation on physicochemical characteristics and fluorescence analysis indicated its potential for medicinal value, among the four different extract of the bark. Methanol extract is showing the good source of bioactive compounds like phenols, flavonoids, tannins, steroids, glycosides etc. Even quantitative estimation of phenol and flavonoids were indicated the presence of sufficient amount of these bioactive compounds in methanol extract, when compared to other extracts of plant. The plant may be a good source of minerals to treat number of diseases that are mainly caused due to the deficiency of those minerals and can be utilized in Ayurvedic medicine system to cure diseases. The antimicrobial property of stem bark of *Ficus krishnae* can is attributed to the presence of high phenol and flavonoid compounds present in extract and their individual or synergistic effect. By above all these parameters we can build up a suitable plant profile which paves way for further studies on the plant for the presence of bioactive compounds and their biological activity.

ACKNOWLEDGEMENT

The authors are thankful to USIC (university science instrumentation centre) to carry out the elemental analysis and also thankful to University Grant Commission (UGC) New Delhi for providing financial support to carry out this research work under Rajiv Gandhi national fellowship (RGNF) scheme.

REFERENCES

1. Hoareau L, Dasilva EJ: Medicinal plants: A re-emerging health aid, Electronic Journal of Biotechnology., 1999; 2:56-70.
2. Ghule ST, Patil DK., Development of medicinal plants, Kisan World., 2001; 28:33-4.
3. Frodin DG, History and concept of big plant genera. Taxon 2004; 53:753-76.
4. Adebayo EA, Ishola OR, Taiwo OS, Majolagbe ON, Adekeye BT., Evaluations of the methanol extract of

Ficus exasperate stem bark, leaf and root for phytochemical analysis and antimicrobial activities, African Journal of Plant Science., 2009; 3:283-7.
5. Chaudhary LB, Sudhakar JV, Kumar A, Bajpai O, Tiwari R, Murthy GV., Synopsis of the genus *Ficus* L. (Moraceae) in India, Taiwania., 2012; 7:193-216.
6. Biswas K, Observations on the systematic position of *Ficus krishnae.* Current Science.,1934;3:424-7.
7. Kirtikar KR, Basu BD. Indian medicinal plants Vol. 3, International Book Distributors, Dehradun India: 2005
8. Chetty MK, Sivaji K, Rao TK., Flowering plants of chittoor district. 2nd ed,Students Offset Printers., Tirupati: 2008-09.
9. Indian Pharmacopoeia, Government of India, Ministry of Health and Family Welfare, Controller of Publication, 4thed, New Delhi, 4(II); 1996, A53-A54.
10. WHO/PHARM/92.559/rev.1, Quality Control Methods for Medicinal Plant Materials,Chase C R and Pratt R S., Fluorescence of Powdered Vegetable drugs with particular reference to Development of a System of Identification, Journal of American Pharmacology Association., 1949; 38:32.
11. Kokoshi CJ, Kokoshi RJ and Sharma FT., Fluorescence of powdered vegetablerugs under Ultraviolet radiation, Journal of Pharmaceutical Association., 1958; 47:715-717.
12. Kokate CK, Khandelwal KR, Pawar AP, Gokhale SB., Practical Pharmacognosy, Nirali Prakashan, 1995 ; 1:11-19.
13. Harborne JB., Methods of extraction and isolation.,In Phytochemical methods, 3rd Ed, Chapman and Hall, London ;1998; 60-66.
14. Raghuramulu N, Madhava Nair K, Kalyanasundaram S., A manual of Laboratory techniques, National Institute of Nutrition, Hydrabad., 2003:175-195.
15. Clinical and Laboratory Standards Institute (CLSI) Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically 6th edition: approved standard M7- A6, CLSI, Wayne (2005).
16. Kumawat U and Shimpi S., Pharmacognostic studies of the Costus Pictus D Don. Journal of herbal medicine and toxicology. 2009;3(1):127-130.
17. African Pharmacopoeia. General methods for analysis Ised, 2: (OAU/STRC) Logos. 1986:123.
18. Musa KY, Katsayal AU, Ahmed A, Mohammed Z, Danmalam UH., Pharmacognostic investigation of the leaves of Gisekia pharmacioides, African Jounal of Biotechnology., 2006:956-957.
19. Kadam PV, Yadav KN, Deoda RS, Narrappanawar NS, Shivtare RS, Patil MJ., Pharmacognostic and Phytochemical Studies on Roots of Agave Americana (Agavaceae), International Journal of Pharmacognosy & phytochemical Reasearch, 2012; 4(3):92-96.
20. Banoti A., Problems relating to the preparation and use of extracts from medicinal plants, Fitoterapia,1980; 51: 5-11.
21. Indrayan, A.K., Sharma, S., Durgapal, D., Kumar, N. and Kumar M., Determination of nutritive value and analysis of mineral elements for some medicinally valued plants from Uttaranchal, Current Science, 2005:1252-1255.
22. Singh, V. and Jain, D.K., Nootan biology Ed 7th, Nageen Prakashan Pvt Ltd, Meerut, India, 2006.
23. Chatterjee, M. N. and Shinde, R., Text book of medical biochemistry Ed 2nd, Jaypee Brother Medical Pub Ltd, New Delhi, India, 1995: 811-846.