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Chemical examination and evaluation of antibacterial, antifungal property of neem (*Azadirachta indica*) and karanj (*Millettia pinnata*) in bhilai – durg region of Chhattisgarh

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Abstract
Medicinal plants act as a great source of economic value all over the people. In present days more chemically drugs are used for the curing disease but it causes adverse effect on body and People are more aware about it. Large number of chemical compounds are found in the plants which is used for certain biological functions and these chemical compounds play a vital role in defending against the pathogenic attack from insects, fungi, viruses etc. Medicinal plants are considered as rich resources of ingredients which can be used in drug development pharmacopoeial, non- pharmacopoeial or synthetic drugs. A part from that, these plants play a critical role in the development of human cultures around the whole world. Moreover, some plants are considered as important source of nutrition and as a result of that they are recommended for their therapeutic values. Some method are used in characterization process are Proximate analysis of leaves of *Azadirachta indica* and *Pongamia pinnata* (*Millettia pinnata*), (Collection of samples Proximate analysis), Pyrolysis extract, Fourier Transform Infrared Spectroscopy analysis, Determination of Zinc concentration and Thermo gravimetric analysis. The leaves of *Azadirachta indica* and *Millettia pinnata* were subjected to proximate analysis. The alcoholic extractives of *Azadirachta indica* and *Millettia pinnata* seeds results reveal how far they differ in their qualities and it gives a finger print out of the sample purity. Ashes gives us the idea of mineral matter contained in the plant which is responsible for pharmacological effect Higher total ash value shows that it had higher mineral content and higher value of acid insoluble ash shows that it has higher digestibility property when the plant is consumed.

Keywords: chemical, *Millettia pinnata*, *Azadirachta indica*, antibacterial, *Pongamia pinnata*

1. Introduction
Medicinal plants are widely used in non-industrialized societies, not least because they are far cheaper than modern medicines. The term “medicinal plant” include various types of plants used in herbalism ("herbology" or "herbal medicine"). It is the use of plants for medicinal purposes, and the study of such uses. The word “herb” has been derived from the Latin word, *“herba”* and an old French word *“herbe”*. Now a day, herb refers to any part of the plant like fruit, seed, stem, bark, flower, leaf, stigma or a root, as well as a non-woody plant. Nearly half the medicines that we use today are of herbal origin, and a quarter contains plant extracts or active chemicals taken directly from plants. Recently, WHO (World Health Organization) estimated that 80 percent of people worldwide mostly used medicinal plants or herbal medicines for some aspect of their primary health care needs. According to WHO, around 21,000 plant species have the potential for being used as medicinal plants. Though the new era has seen advances in chemistry, which have paved the way to reproduce the active ingredients found in plants, but plants still will continue to have the medicinal importance in their own right. Their active components may be slightly modified to improve their efficacy or to reduce undesirable side effects, but they are still the basis of drugs that are vital for treatment of disorders such as cancer or diabetes, one such classical example is the modified form of the compound acetylsalicylic acid more commonly known as aspirin was the first modern drug that was born from the nature’s medicine, the bark of the willow tree. It is no accident that plants play an important role in contemporary pharmacological research: the compounds that have the medicinal applicability are those that the plant itself uses to survive. There are more than 100,000 of these active compounds have been found in the plant world, because of their complex and diverse chemical structures, they are the basis of many medicines. *Azadirachta indica* and *Millettia pinnata* are the common medicinal plants which is commonly known as Neem and Karanj. It is widely distributed throughout India. Due to its beneficial properties, it is used from the ancient time. it is considered widely beneficial in variety of diseases but mainly it is used to cure worm infestation and its benefit was found in Aaurveda. It is also used as medicine in Homeopathy.

a) *Azadirachta Indica*
Neem (*Azadirachta indica*). Description and Medicinal Uses of *Azadirachta indica* (Neem). Classification of Neem (*Azadirachta indica*). All parts of Neem tree used as anthelmintic, anti-fungal, anti-diabetic, antibacterial, antiviral, contraceptive and sedative.

Sicientific classification of *Azadirachta indica*
Common name- Neem
Kingdom- Plantae
Order - Sapindales
Family - Meliaceae
Genus - Azadirachta

Species - *Azadirachta indica*

Azadirachta indica belongs to family Meliaceae. It is a tree native to India, Nepal, Pakistan, Bangladesh and Srilanka. It grows in tropical and subtropical region. It can grow in regions with annual rainfall below 400 mm. It is emergress, branches are wide and spreading one. It is a shade giving tree and survives in dry coastal areas, south districts of India and Pakistan. Height of the tree is about 15-20 metres.

Fig 1: Tree, leaf and fruit *Azadirachta indica* (Neem)

b) *Pongamia pinnata*

Pongam tree is found to be one of the richest trees of India. The name *Pongamia pinnata* is derived from the word pinnata that refers to the pinnate leaves. It belongs to family Fabaceae. It is native in tropical and temperate Asia. It is grown in Indian subcontinent China, Japan, Malaysia, Australia and Pacific islands. It is now called *Millettia pinnata* as it is moved to the genus Milletia only recently.

Scientific classification of *Pongamia pinnata*

Kindom	-	Plantae
Order	-	Fabales
Family	-	Fabaceae
Genus	-	Millettia
Species	-	M. pinnata

Pongamia pinnata is named as Karanj in Hindi, Honge in Kannada, Pungai in Tamil, Kanuga in Telugu, Karach in Bengali, Naktanala in Sanskrit.

Fig 2: *Pongamia pinnata* flower and tree for analysis.

Millettia pinnata belongs to pea family. It is deciduos for short periods. It grows to about 50-80 feet in height and has a large canopy which spreads wide. It has straight trunk 50-80 cm in diameter with rough and grey brown bark. Leaves are imparipinnate, alternate and are short stalked, rounded at the base, ovate along the length, obtuse acuminate at the apex and not toothed at the edges. Leaves have five, seven or nine oval shaped leaflets with pointed tips.

2. Methods & Methodology

2.1 Proximate analysis of seeds of *Azadirachta indica* and *Millettia pinnata*

2.1.1 Collection of samples

The studies were undertaken on medicinal plants *Azadirachta indica* and *Millettia pinnata* of family Meliaceae and Fabaceae respectively. The choice of plant parts were leafs of *Azadirachta indica* and *Millettia pinnata* which was collected from were collected from Bhilai-Durg region of Chhattisgarh, India and was taxonomically authenticated. A care was taken to select healthy plants and the plant parts for the study were collected fresh and dried for a week to be involved in the proximate analysis.

Leaf of Karanj **Dry leaf** **Powder form of leaf**

Fig 3

Fig 4

2.1.2 Proximate analysis

Extractive values

About 5g of the dried and finely coursed powder is mixed with 100 ml of 90 % ethanol in a closed flask. The flask was frequently shaken during the first 6 hours and allowed to stand for 18 hrs. Then the mixture was rapidly filtered to minimize the loss of ethanol and 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish. The residue was dried at 1050 C for minutes and then weighed. The procedure was performed twice more from the filtrate.

Ash values

Total ash and sulphated ash values

A silica crucible was heated for about 30 min to red hot and cooled in a desiccators to note down its weight. About 3 g of the powdered sample were weighed and then dried at 100-105ºC for 1 hr and ignited to constant weight in a muffle furnace at 600-625 ºC until a carbon free ash is formed. The crucible was allowed to cool in a desiccators after each ignition and care was taken to avoid catching fire. The weight of the carbon free ash was determined. The procedure was repeated to obtain a standard deviation to ensure consistency and then tabulated. The same procedure was carried out adding dilute sulphuric acid to determine the yield of sulphated ash.

Acid Insoluble ash

About 1g of the total ash (from total ash) was boiled with 25 ml of 2M hydrochloric acid for 5 min. The acid insoluble was separated by filtration on an ash less filter paper in Gooch crucible. The content on the ash less filter paper was washed with hot water and ignited and then weighed to obtain the percentage of ash with reference to the air dried samples.

Water soluble ash

About 1g of the total ash was boiled with 25 ml of water for 5 min and then filtrated to retain the insoluble matter on ash less filter paper. The content was ignited for 15 min at a temperature not exceeding 4500 ºC then weighed. The difference between the amount of ash subjected and weight of insoluble ash was accounted as the water soluble ash value.

Loss on drying About 10 g of each specimen under study were accurately weighed and transferred to a charred china dish which was known for its weight and kept in a hot oven at 100-1050 ºC for an hour. Then the sample was weighed along with china dish to deduct the actual weight of tarred china dish. The weight of the powder was noted to calculate the percentage loss on drying with reference to air dried specimen.

2.1.3 Pyrolysis extract of *Azadirachta indica* and *Millettia pinnata*

Experimental set up and procedure for pyrolysis The experimental set up consist of a heating chamber in which the dried leaves of *Azadirachta indica* and *Millettia pinnata* are placed separately turn by turn and that is then closed very tightly so as to avoid any leakage of gas as the result of pyrolysis. A pressure cooker of 3 liter capacity is used as a heating chamber as Aluminium used here is one of the better conductors of heat. The heating is done by 1000 W Ni-chrome heating coils which are attached at the periphery of pressure cooker and heating is done to 6000ºC. The entire arrangement is packed inside an Aluminum vessel with a lining of asbestos fibers which help in insulation and do not permit the loss of heat. A screw fitting is given at top of cooker to make it air tight and for the exit of fuming gases there is one suitable passage at the top of the cooker. Also a temperature sensor is provided which continuously monitors the temperature inside the vessel. The outlet of the reactor is connected to a condenser which condenses the gases coming of the outlet. Just at the other end of the condenser a measuring cylinder is placed where the gases being condensed is collected. The dried Seeds are fed in to the heating chamber and it is closed very tightly using screw and bolt. Once the heating is started and after reaching a suitable temperature the reaction begins and the vapours that are released comes out of the reactor outlet which is connected to the condenser where the vapours are condensed and collected in the measuring cylinder Most of the non-condensable vapours are simply released. The product mainly consists of pyrolytic oil and water which then is separated based on density difference. Fourier Transform Infrared Spectroscopy analysis of *Azadirachta indica* and *Millettia pinnata*, FTIR spectrum helps to identify the functional group present in *Azadirachta indica* and *Millettia pinnata*. FTIR spectra are recorded in KBr by sophisticated computer controlled FTIR Perkin Elmer spectrometer with He- Ne Laser as reference. The pyrolysis extract of sample *Azadirachta indica* and *Millettia pinnata* were scanned at room temperature and a spectral range of 4000-400 cm^{-1}. Determination of Zinc concentration in Ocimum sanctum leaves Analysis of Zinc concentration in the pyrolysed extract of *Azadirachta indica* and *Millettia pinnata* seeds were determined by Atomic Absorption Spectrophotometer Perkin Elmer 2380 model using suitable hollow cathode lamps. Thermo gravimetric analysis of *Azadirachta indica* and *Millettia pinnata* leaves TGA were performed with TGA 4000, Pyris 6 TGA. Weight of *Azadirachta indica* and *Millettia pinnata* leaves taken was 6.910 mg. These were loaded separately on quartz pan and

mounted in instrument. Initial conditions of temperature were 300ºC and switch the gas to N_2 at 20 ml/ min. Temperature programming were heating rate from 300ºC to 4000ºC at 100ºC in nitrogen and hold for 1 min at 300ºC.

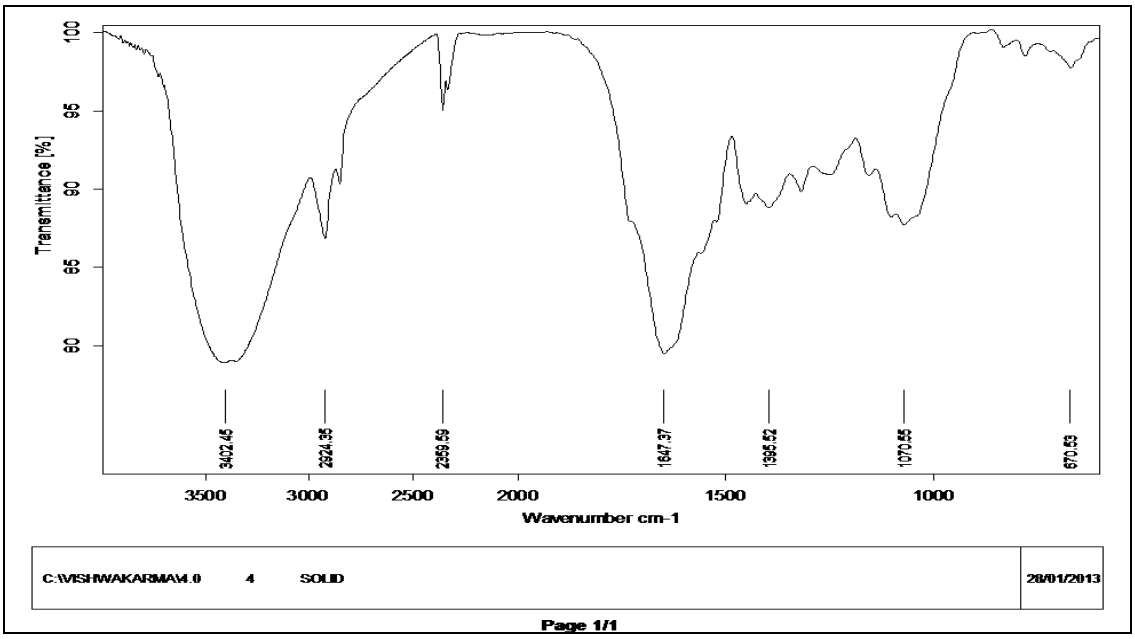

Fig 5: FTIR spectra of seeds of *Azadirachta indica* (Neem)

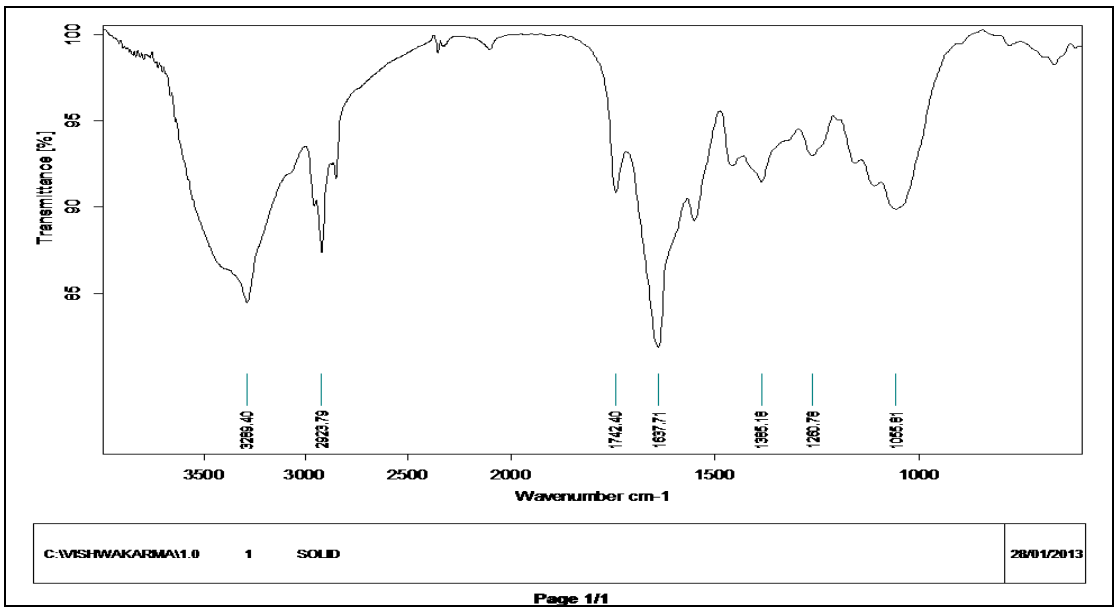

Fig 6: FTIR spectra of leaves of *Pongamia pinnata* (Karanj)

2.2 Anti-bacterial testing

Antibacterial activity was measured using agar dilution technique. Briefly, the methanol extracts were dissolved in dimethyl sulfoxide (DMSO, Merck) and serially diluted in molten Mueller Hinton Agar (MHA, Sigma) in petridishes (100 mm×15 mm) to obtain final concentrations: 100, 50, 25 and 12.5 µg/ml. The solvent did not exceed 1% concentration and did not affect the growth of the organisms. All bacterial strains were grown in Mueller Hinton Broth (MHB, Sigma) for 4 h at 37°C. Bacterial suspensions with 0.5 McFarland standard turbidity, which is equivalent to 108 cfu/ml, were prepared by dilution with Mueller Hinton broth. The diluted inoculum was added to a Steer's replicator calibrated and incubated for 24 h at 37 °C. After incubation, all dishes were observed for microbial inhibition by the disc diffusion method [35]. Streptomycin sulphate (10 µg mlG) used as positive control and methanol solvent (100 µg mlG) used as negative control. The antibacterial assay plates were incubated at 37°C for 24 hours. The diameters of the inhibition zones were measured in mm.

2.3 Antifungal Activity

The antifungal activity was tested by disc diffusion method. The potato dextrose agar plates were inoculated with each

fungal culture (10 days old) by point inoculation. The filter paper discs (5 mm in diameter) impregnated with 100 µg mlG concentrations of the extracts were placed on test organism-seeded plates. Methanol was used to dissolve the extract and was completely evaporated before application on test organism-seeded plates. Blank disc impregnated with solvent methanol followed by drying off was used as negative control and Nystatin (10 µg discG) used as positive control. The activity was determined after 72 h of incubation at 28°C. The diameters of the inhibition zones were measured in mm.

Table 1: Proximate analysis on leaves of *Azadirachta indica* (Neem) and *Millettia pinnata* (Karanj)

S. No.	Experimental studies	Neem leaves %w/w	Karanj leaves %w/w
1	Total ash value	12.81	6.97
2	Water soluble ash	55.3	45.92
3	Acid insoluble ash	9.66	10.08
4	Sulphated ash	10.86	6.86
5	Loss on drying	5.75	4

Table 2: Proximate analysis of Karanj (Milletia pinnata), Neem (*Azadirachta indica*)

Extractive values	Specimen	Colour of the residue	Extractive % w/w
Alcoholic extractives	*Millettia pinnata*-leaves	Green	5.41
Alcoholic extractives	*Azadirachta indica*-leaves	Dark brown	3.05

3. Result & Discussion

The seeds of *Azadirachta indica* and *Millettia pinnata* were subjected to proximate analysis and results were as mentioned in Table-1. The alcoholic extractives of *Azadirachta indica* and *Millettia pinnata* seeds were 4.04% w/w and colour of the residue were light brown. The results reveal how far they differ in their qualities and it gives a finger print out of the sample purity. Ashes gives us the idea of mineral matter contained in the plant which is responsible for pharmacological effect Higher total ash value shows that it had higher mineral content and higher value of acid insoluble ash shows that it had higher mineral content and higher value of acid insoluble ash shows that it has higher digestibility property when the plant is consumed. The current study was initiated because of the increasing resistance to antibiotics including bacteria and fungi. Plant extracts and compounds are of new interest as antiseptics and antimicrobial agents. As a result, the antimicrobial activity of different medicinal plant parts extracts of four plants was screened against the most common pathogens. In general, methanol leaf extracts of the selected plants appeared to be effective source of active antimicrobial agents. The present study was undertaken with a view to identify the functional groups present in the leaves and seeds of the medicinal plants taken with the help of FTIR analysis. It helps to identify the chemical constituents, elucidate the chemical structure and also effort was taken to understand the significance of functional groups as bio active constituents for the treatment of various diseases. The very strong absorption band observed around 3373-3422 cm^{-1} may be due to the presence of bonded N-H/C-H/O-H stretching of amines and amides The very strong absorption band observed in 1600-1660 cm^{-1} region indicates the presence of amino acids. The strong absorption band observed between 3200-3400 cm^{-1} indicates the presence of polymeric hydroxyl derivatitives. Vibration of N-H shows the presence of primary amine. The band observed at near 2848 cm^{-1} represent C-H symmetric stretching of methylene group in aliphatic. C=C stretching region falls with the range 1511-1561 $cm^{-1.}$ Similarly the Chelated C=O stretching vibrations lie towards the lower wave number side that is within the range 1621-1635 cm^{-1}.

There is no absorbance in between the region 2220-2260 cm^{-1} indicates that no cyanide groups in all the extracts of the medicinal plants taken. These results exhibit that the medicinal samples taken for the study does not contain any toxic substances.

Presence of C=O,C-H,C=C and C-O,C-C and C-O bonding structures were responsible for the presence of alkyl groups, methyl groups, alcohols, ethers, esters, carboxylic acids, anhydrides and deoxyribose (Dukor *et al* 2002 and Sohrabi *et al* 2005). The more intense bands occurring at 3419 cm-1,2927,2853,1633,1421,1260,1073,816and 635 cm^{-1} corresponding to O-H/N-H,C_H,C-O and C-Cl/C-CS stretching / bending vibrations respectively indicate the presence of amino acids, alkenes, nitrates, ethers, organic halogen compounds and carbohydrates in plants.

Carboxylic acid present in the medicinal plant serves as main pharmaceutical product in curing ulcers, jaundice, headache, stomatitis, hemicranias, fever, pain in liver, wound in cattle, treatment of edema and rheumatic joint pains.

Amines, amides and amino acids are the main groups of protein synthesis and herbs serves as herb oil and hair tonic. Sulphur derivative compounds were used as disinfectants and dermal cream. Polysaccharides, carbohydrates, chlorates and nitrates play the role of the disinfectants.

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5. References

1. Muhamed Mubarack H, Doss A, Dhanabalan R, Venkataswamy R. In-vitro antimicrobial effects of some selected plants against bovine mastitis pathogens. Hygeia. J Drugs Med. 2011; 3:71-75.
2. Mustafa MR, Mahmood AA, Sidik K, Noor SM. Evaluation of wound healing potential of Ageratum leaf extract in combination with honey in rats as a minor

model. Int. J Mol. Adv. Sci. 2005; 1:406-410.
3. Neeraj T, Madivi A. Studies on antibacterial activity of methanol plant extracts. Int. J Phytomed. 2000, 2011; 3:549-556.
4. African Traditional Medicine. A Dictionary of Plant Uses and Application. Medipharm Scientific Publishers, Stuttgart, 421-422.
5. Okwori A, Dina C, Jumaid S, Okeke I, Adetunji J, Olabode A. Antibacterial activities of Agerutum conyzoides on selected bacterial pathogens. Int. J Microbiol. 2013; 4(1):1-4.
6. Oliver-Bever B. Medicinal plants in tropical West Africa. Cambridge, London. Osho A, Adetunji T. Antibacterial activity of the essential oil of Ageratum conyzoides L. Asian J Sci. Technol. 2011; 2:1-5.
7. Oskay M, Oskay D, Kalyoncu F. Activity of some plant extracts against multi-drug resistant human pathogens. Iranian J Pharm. Res. 2009; 8:293-300.
8. Rabe T, van Saden I. Antibacterial activity of South African plants used for medicinal purposes. J Ethnopharmacol. 1997; 56:81-87.
9. Raghavendra R, Mahadevan GD. In vitro antimicrobial activity of various plant latex against resistant human pathogens. Int. J Pharm. Pharm. Sci. 2011; 3:70-72.
10. Assay for antimalarial and amoebicidal activities; Methods in Plant Biochemistry, Bd. Academic Press Limited, Great Yarmouth, Norfolk. 1991, 135-152.
11. Evans CE, Banso A, Samuel OA. J Ethno pharmacology, 2002; 80:21-24.
12. Chaurasia SC, Jain PC. Indian J Hosp Pharm. 1978; 166-68 [14] Joe, B., M. Vijaykumar, and B. R. Lokesh. J Food Sci. Nutr. 2004; 44:97-111.
13. Fang JY, Hung CF, Chiu HC, Wang JJ, Chan F, Pharm J. Pharmacol. 2003; 55:1175.
14. Tang W, Eisenbrand G, J Plant Research 1992, 401-415.
15. Chander H, Kulkarni SG, Berry SK. J Insect Sci. 1991; 5:220-222.
16. Lee HS, Ahn YJ. J Agric. Food Chem. 1998; 46:8-12
17. Nir Y, Potasman I, Stermer E, Tabak M, Neeman I. J Pub Med. 2000; 5:94-97.
18. Limtrakul P, Anuchapreeda S, Buddhasukh D. J Pub Med. 2004; 4:13
19. Luthra PM, Singh R, Chandra R. Indian J Clin. Biochem., 2001; 16:153-160.
20. Chopra RN, Gupta JC, Chopra GS. Indian J Med Res. 1941; 29:769-772.
21. Maslin BR, Miller JT, Seigler DS, J Systematic Botany. 2003; 16(1):1-18.
22. Dafallah AA, Mustafa Z Am. J Chin. Med. 1996; 24:263-269.
23. Shah BH, Safdar B, Virani SS, Nawaz Z, Saeed SA, Gilani A. J Pharmacol. 1997; 29:251-255.
24. Amos S, Akah PA, Odukwe CJ, Gamaniel KS. Wambede C J Phytother. Res. 1999; 13:683-685.
25. Tezuka Y, Honda K, Banskota AB, Thet MM, Kadota S, J Nat. Prod. 2000; 63:1658-1664.
26. Chang ST, Wu JH, Wang SY, Kang PL, Yang NS, Yur LF. J Agri. Food. Chem. 2001; 49:3420-3424.
27. Ramya Krishna PS, Bhaduri Lavanya, Pulla Sireesha S. Nagarjuna Y. Padmanabha Reddy, Der Pharmacia Sinica, 2011; 2(6):17-22 [30] U Devi. Ind. J Pharm Sci. 1996; 34:927-932.
28. Manwar JV, Mahadik KR, Paradkar AR, Takle SP, Sathiyanarayanan L, Patil SV. Der Pharmacia Sinica. 2012; 3(1):41-46.
29. Satyapal Singh. Babeet Singh Tanwer and Moinuddin Khan, Advances in Applied Science Research. 2011; 2(3):47-52.
30. Chatterjee A, Pakrashi SC. The Treatise on Indian Medicinal Plants. 1995; 4:208-212.
31. Bone K. Clinical Applications of Ayurvedic and Chinese Herbs. Australia: Phytotherapy Press. 1996, 137-141.
32. Chopra RN, Chopra LC, Handa KD, Kapur LD, editors. Indigenous Drugs of India. 2nd ed. Kolkata: M/S Dhar VN & Sons; 1982. [36]. Zhao TF, Wang X, Rimando AM, Che C. Med. 1991; 57:505.
33. Ambati Srinath, Jyothi V, Asha Jyothi V, Pharmacological, pharmacognostic and phytochemical review of *Azadirachta indica* and *Millettia pinnata*, IJPT, 2010; 2(4):525-539.
34. Arora Neha and Rai Shashi Pandey. Celastrus paniculatus an endangered Indian medicinal plant with miraculous cognitive and other therapeutic properties, an overview, Int J Pharm Bio Sci. 2012; 3(3):290-303.
35. Bhanumathy M, Chandrasekar SB. Chander Uma, Somasundaram T. Phytopharmacology of Celastrus paniculatus, an overview, ITPSDR, 2010; 2(3):176-181).
36. Bhowmik S, Chowdhury SD, Kabir MK, Ali MA, Chemical composition of some medicinal plants products of indigenous origin, Bangl.vet. 2008; 25:32-39.
37. Bobade SN, Khyade VB. Detail study on the properties of *Pongamia Pinnata* (Karanja) for the production of biofuel, Research Journal of Chemical Sciences. 2012; 2(7):16-20.
38. Jain Ritesh, Jain Sanmati K. Traditional medicinal plants as anti cancer agents from Chhattisgarh, India; An overview, International Journal of Phytomedicine. 2010; 2:186-196.
39. Joseph Baby, Nair Vrundha M. Ocimum sanctum linn (Holy basil) Pharmacology behind its anti-cancerous effect, Int J Pharm Bio Sci. 2013; 4(2):556-575.
40. Joshi Bishnu, Sah Govind Prasad, Basnet Buddha Bahadur, Bhatt Megh raj, Sharma Dinita, Krishna Subedi, Janardhan Pandey and Rajani Malla. Anti microbial property of alcoholic extracts of different medicinal plants against different pathogenic micro organisms, Journal of Microbiology and Antimicrobials. 2011; 3(1):1-7.
41. Katekhaye S, Kale MS, Laddha KS. Development and validation of an HPLC method for Karanjin in *Pongamia pinnata* linn. leaves. Indian J Pharm Sci. 2012; 74:72-5.
42. Mohammad Mehdi Sadeghian and Forough Mortazaienezhad, Investigation of compounds from *Azadirachta indica* (Neem), Asian Journal of Plant sciences. 2007; 6(2):444-445.
43. Ogbuewu I. Odoemenam P, Obikaonu HO., Opara MN, Emenalom OO. Uchegbu MC, *ET AL*., The growing importance of Neem (*Azadirachta indica* A. Juss) in agriculture, industry, medicine and environment: A review. Research Journal of Medicinal Plant. 2011; 5:230-245.
44. Prasad K, Janve1 B, Sharma RK, Prasad KK.

Compositional of traditional medicinal plants: chemo metric approach, Archives of applied science research. 2010; 2(5):1-10.
45. Rathore JS, Upadhyay Mohit. Investigation of Zinc concentration in some medicinal plant leaves, Research Journal of Pharmaceutical Sciences. 2(1):15-17.
46. Schaaf Otmar, Jarvis Andrew P, Andrew van der Esch S, Germina Giagnacovob, Neil J Oldham. Rapid and sensitive analysis of Azadirachtin and related triterpenoids from Neem (*Azadirachta indica*) by high-performance liquid chromatography–atmospheric pressure chemical ionization mass spectrometry, Journal of Chromatography A. 2000, 886.
47. Shafqatullah, Muhammad Khurram, Asadullah Khaliqurrehman, Farhat Ali khan. Comparative Analysis of Ocimum santum Stem and Leaves for phytochemicals and inorganic constituents, Middle-East Journal of Scientific Research. 2013; 13(2):236-240.
48. Sinha MK, Patel DK, Kanungo VK. Medicinal plants used as antidotes in northern part of Bastar district of Chhattisgarh Journal of Ecobiotechnology. 2012; 4(1):58-60.