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STUDY OF CHEMICAL CHARACTERIZATION OF NIMBA STEM BARK FOR REMEDIAL PURPOSE

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Abstract-
This is one of the important medicinal plants from ancient times. Rural population use its tender branches as tooth brush even today. The tree trunk is used as lumber to make furniture, leaves, bark, resin (gum), flowers, and seeds are used for medicinal purpose. The Neem bark contains mimbin (0.04%), mimbinin (0.01%), mimbidin (0.04%), mim bosterol (0.03%), essential oil (0.02%) tannin (6.0) and a bitter principle, margosine. Various parameters were analysed in the present study i.e.moisture content, water soluble extractive value, alcohol soluble extractive value, total ash, pH, phytochemical tests i.e. alkaloid, tannin, resin, saponin, flavonoids, carbohydrate, protein and HPTLCtest. The results of study showed that moisture content and total ash value were found 6.76 g. and 7.641 g. respectively. The value of acid insoluble ash and water soluble ash were found 16.18% and 14.156 % respectively. The percentage of alcohol soluble extractive value and water soluble extractive values were found 14.85 % and 11.416 % respectively. It was observed that the phytochemical parameters i.e. saponin, tanin, carbohydrate were also present in the samples. The study revealed that the drug was authentic and genuine. The studied parameters can be used as diagnostic tools for identification of drug. Neem leaves should be utilized as remedial i.e. antiseptic and anti-microbial purpose. The neem may be used as an interal medicine to lower blood glucose level, treat peptic ulcers and kill intestinal parasites.

Keywords- Neem Leaves, Nimba Stem Bark, Azadirachta Indica, pH, Wagner's Test, HPTLC, Remediation

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I. INTRODUCTION

Its status as national tree justifies the significanceof medicinal and commercial value. This is one of the important medicinal plantsfrom ancient times. Rural population use its tender branches as tooth brush even today. Neem leaves are utilized for antiseptic and anti-microbial purpose.

Neem (Nimba) Azadirachta indica

Thetree trunk is used as lumber to make furniture, leaves, bark, resin (gum), flowers, and seeds are used for medicinal purpose. Theextract from bark leaves fruits and seeds are applied topically as antiseptic, antibacterial, antiviral, antifungal and medicine. It is also natural astringent. The neem is used as an interal medicine to lower blood glucose level, treat peptic ulcers and kill intestinal parasites. This is one of the large evergreen trees grown in India. It may live up to 200 years and grow up to 125 feet tall.

Synonyms –

Sansk	:	Arista, Picumarda
Assam	:	Mahanim.
Beng	:	Nim, Nimgacha
Eng	:	Margosa Tree
Guj	:	Kadvonimbo
Hindi	:	Nim, Nimb
Kan	:	Nimba,Bevu, Oilevevu, Kahibevu
Mal	:	Veppu, Aruveppu .

Mar : Blantanimba, Nimba, Kadunimb
Ori : Nimba
Punj : Nimba, Bakam, Nim.
Tam : Veppai, Vembu
Tel : Vemu, Vepa
Urdu : Neem

Scientific Classification –
Kingdom : Plantae
Division : Magnoliophyta
Order : Sapindales
Family : Meliaceae
Genus : Azadirachta
Species : A. Indica

Chemical Constituents –
Bark contains mimbin (0.04%), mimbinin (0.01%), mimbidin (0.04%), mim bosterol (0.03%), essential oil (0.02%) tannin (6.0) and a bitter principle, margosine (Bhandari & Mukerji, 1959). Desacety linimbin isolated from seeds & bark (Narayan & lyer, 1967) quercetin from leaves(Bark & Chakroborty, 1968).

Part Used: Rootm, stem barl, leaves, fruits, seeds & seed oil

Formulations: Nimbadi Kvatha curna, Nimabdi curna, Pancanimba curna, Pansatika Guggulu grhrta, Pathyadikwatha (sandhga) curna, Sudersana curna.

Therapeutics uses: Vrana, Kustha, Prameha, Kandu, Krmiroga, Jvara, Daha, Raktapitta

Medicinal Uses: Stem is bitter coolent,antheminthic, antiperiodic and as tringent. It is a powerful blood purifier. It rehabilitates vitiated pitta and kapha and is useful in intestinal worms rehabilitates, impurity of blood, eye disease, intermittent fevers, thirst, vomiting, general debility, diabetes, leprosy, skin diseases, ulcers and inspect poisons. Seed oil is used as applications in ulcers, chronic skin diseases itch and rheumatism.

II. REVIEW OF LITRATURE

Gomase et.al (2011) studied on phytochemical evaluation and analgesic activity of fresh juice of young stem (tender) bark of Azadirachta Indica A. Juss. The effective dose of the extract for analgesic activity was calculated from dose-response curve by using the Eddy's hot plate method and heat conduction method response in rats. In both of the cases diclofenac sodium was used as standard drug. In both of Eddy's hot plate method and heat conduction method response in rats writhing response method; the intraperitoneal administration of fresh juice of juice of young stem (tender) bark of Azadirachta indica A. Juss (200mg/kg, 300 mg/kg and 500 mg/kg) induced a significant analgesic activity in a dose-dependent manner respectively. The plant may have the phyto-constituents which inhabit cyclooxygenase enzyme or act on central opioid receptors. Insecticidal effects of various neem preparations against some insects of agricultural and public health concern by Achio et. al (2012). Neem (Azadirachta indica) has some medicinal and pesticidal properties resulting from its various active components, including azadirachtin. The lethal effect was more pronounced with the seed extract (40-55%), followed by the leaf extract (30-45%), the stem extract (30-40%) and the root extract (10-30%), as compared to the (distil water) which registered 0% mortality. The termites and the weevils were seen to be more susceptible to the various extracts, compared to the cockroaches and mosquito larves. Oil extracted from the neem seed kernel showed even greater lethal concentration, in all cases, being 0.50% V/v. Again the termites and the weevils responded faster, recording total deaths within 2-5 minutes,

compared to the cockroaches and the mosquito larvae where total deaths were experienced only after 30-90 minutes. It was also found out that there was a direct relation between the concentration and degree of lethal effectiveness of the oil. The neem, especially the seed oil, has great potential as natural biocide against termites and weevils.

Akin-Okaniae et.al (2013) reported on antimalarial effect of neem, stem bark extracts on plasmodium berghei infected in the pathology and treatment of malaria. The study was designed to carry out the antimalarial screening of neem extracts using Plasmodium berghei infected albino mice. Albino mice free from infection were experimentally infected with Plasmodium berghei. The animals showed detectable parasitemia on day 4 post- infection with about 30 % of parasitemia before death of animal was recorded. Different parts of neem (leaf, stem bark and seed) were extracted with methanol and their efficacy tested on Plasmodium berghei infected albino mice using the 4-day suppressive test and secondary biological assessment procedures. The lethal median dose (LD50) recorded for neem leaf and stem bark extracts were 31.62 and 489.90 mg/kg body weight respectively. Neem leaf and stem bark extracts reduced the level of parasitemia in infected mice by about 51 - 80% and 56 - 87% respectively.

Non-wood products are known to have antiallergenic, antidermatic, antecedent, antifungal, anti-inflammatory, antipyorrhoeic, antiscabic, cardiac, diuretic, insecticidal, larvicidal, nematicidal, spermicidal and other biological activities. Because of these activities neem has found enormous applications making it a green treasure reported by Girish and Bhat (2008).

Khalid et.al. (1989) found that neem extracts similarly affected the survival of salmonella. The leaf, stem or root bark extract showed similar effects in reducing egg hatch or larval survival suggest that the extracts contain similar active components possibly in similar concentrations and probably possess the same mechanisms of action.

NWOSU et.al. (2006) studied on in-vitro anthelmintic efficacy of crude aqueous extracts of neem(Azadirachta indica) leaf, stem and root on nematode. The reduction in larval survival due to the extracts was similar to that produced by albendazole. In general, the aqueous extract of neem leaf was more efficacious in limiting nematode larvae survival and in-vitro egg hatch. The results confirm the folkloric claims that neem has anthelmintic effect and thus suggest its possible usefulness as an anthelmintic. By Ayuba et. al (2011) crude Phytochemicals in the Foliage and stem-bark of Azadirachta indica. It was further revealed that there were no significant interactions between the tree parts (foliage and stem-bark) (at $P > 0.05$) for the phytochemicals (alkaloid, flavonoid, saponins, and total Phenols) that were quantitatively screened. In addition, there were significant differences in the amount of the phytochemicals in the species. Generally, there were more concentration of total phenols (2.53 ± 0.36 g/g) than that of the other phytochemicals determined (alkaloids, flavonoids and saponins that did not differ significantly with mean values of 0.11 ± 0.01 g/g, 0.14 ± 0.03 g/g, and 0.31 ± 0.06 g/g respectively). The results implied that the species had potentials in pharmaceutical, agrochemical and allied industries.

Abu and Uchendu (2011) reported on effect of aqueous ethanolic extract of hymenocardia acida stem bark on oestrous cycle of albino rats. It is well known that some plant preparations play important role in fertility regulation. Oestrous cycles of albino rats showing regular cycles were monitored daily by vaginal lavage. The length of oestrus cycles and duration of each phase of the cycle were recorded. We observed that the aqueous ethanolic extract of Hymenocardia acida stem bark caused an irregular oestrous cycle characterized by prolonged diestrus phase. It is concluded that the extract caused a loss of cyclicity in female albino rats.

Kabir and Muhammad (2010) studied on comparative studies of seed oil extract, leaves and stem bark powders of Azadirachta indica Linn (Meliaceae) on adults Callosobruchus maculatus (Coleoptera Bruchidae). In this study was observed that at different concentration of the three extract at 0.08g. 0.17ml

0.25ml of seed oil and 0.08g,0.17ml and 0.25g of leaves powder affected the oviposition, fecundity of adults, emergence of young ones and frequency hatched larvae. In the case stem bark powder little activity was observed at all the three different replications investigated. Exposure of insects/larvae to 0.08ml of seed oil and 0.08g of leaves powder show the highest activity among the different concentration used. It can be seen from the above inference deduced that Neem tree extract and powders are recommended for the storage of the black beans Phaseolus vulgaris Linn against Callosobruchus maculatus.

Study was undertaken to observe the effect of neem oil, on the microscopic structure of testes in mature male albino rats and the associated changes in the serum levels of male reproductive hormones. The animals were divided in different groups as A1 = treated males at low dose (0.6 mL of neem oil/animal), A2 = treated males at high dose (1.2 mL of neem oil/animal), A3 = controls for group A1 (corresponding dose of peanut oil) and A4 = controls for A2 (corresponding dose of peanut oil). Animals were kept under observation for a period of six weeks. At the end of this period animals were anesthetized, blood was removed by cardiac punc-ture and sacrificed. Testes were removed and fixed in10% formal saline for microscopy and methanol for HPLC purpose by Shaikh et. al (2009).

III. MATERIAL AND METHODS

The following materials i.e. glassware and instruments were used in the present work.

S.No.	Glass ware	Instruments
1.	Capillary tube	Compound microscope
2.	Slides	Desicatar
3.	Condenser	Digital pH meter
4.	Conical flask	Hot air oven
5.	Funnel	Micro pipette
6.	Glass rod	Shake
7.	Iodine flask	Water bath
8.	Measuring cylinder	Weighing machine
9.	Petridish	Soxhlet extraction unit
10.	Pipette	HPTLC
11.	Reagent bottle	
12.	Test tube	

The parameters were anlysed in the present study i.e.moisture content, water soluble extractive value, alcohol soluble extractive value, total ash, pH, phytochemical tests and HPTLC test.

i. Determination of Moisture Content:

Took3 petridish and weighed accurately (preweight of petridish), then placed about 5 gm. Drug powder in petridishes. Then allowed to dry in hot air oven for 5 hours 105°c, after 5 hours allowed to cool in desiccator, then weighed the petridish with drug. After again allowed to dry for 30 minutes at 105°c then cooled and noted down the final reading.

ii. Determination of water soluble extractive value:

Took an iodine flask, added 100 ml water and 2 gm. drug, and kept the flask for centrifugation for 6-7 hours. and allowed it to stand for 18 hours, then filtered with no.42 (Whatman) filter paper and 100 ml of filtrate solvent was poured in each petridish (3 petridishes), and kept it in water bath for evaporation. Then calculate the percentage of water soluble extractive with reference to the air dried drug.

iii. Determination of alcohol soluble extractive:

Same method was adopted as for no. 2 parameter.

iv. Determination of total Ash value:

Took 6 crucible dish/silica dishes and weighed all of these, then added 2-2 gm. sample powder in each crucible, then allowed to heat in muffle furnace for 6 hours at 450°C. Next day these crucible were allowed to cool then weight was noted down. After that again kept the crucible in muffle furnace and again weighed after cooling. This process was repeated till constant was obtained. Calculate the percentage of ash with reference to the air dried drug.

v. Determination of pH value:

Took 5 gm. sample powder in an iodine flask and added 50 ml. distilled water and allowed for 2 hours. Then filtered with 42 no. Filter paper and took the pH of the filtrate with electronic pH meter.

vi. Phytochemical tests:

Wagner's test for alkaloid:

1ml of alcoholic extract is acidified with a HCL. Added few drop of Wagner's reagent. Absent of alkaloid were indicated by formation of yellow or brown colored ppt.

Test for tannins:

Took 1-2 ml of aqueous extract, few drops of 5% FeCL3 is added. A brown colour indicates the presence of tannins.

Test for resin:

1ml of aqueous extract was dissolved in 2m1 of acetone and then solution was poured in tube containing 2 ml of distilled water. Appearance of turbidity indicates the presence of resins.

Test for saponin:

Took 5ml of aqueous extract, a drop of NaHCO3 is added and shaked vigorously and left for few minutes. Formation of honey comb indicates the presence of saponin.

Test for flavonoids:

Took 5-10 drops of alcoholic extract, then 5-10 drop of dilute HCL was added and piece of Mg metal were added. Pink, brown colour is developed, indicates the presence of flavonoids.

Test for carbohydrate:

Took 2ml of aqueous extract is taken in a test tube and 1m1 of mixture of Fehling solution A and B is added andboiled for few minutes. Formation of brick ppt. Indicates presence of carbohydrate.

Burette test for protein:

Took 1m1 of hot aqueous extract, 5-8 drops of 10%NaOH solution was added, and then added 1-2 drops of 3% Cuso4 solution. A violet colour indicates the presence of protein.

vii. High Performance Thin Layer Chromatography (HPTLC):

Test solution:5 gram of coarsely drug was placed into flask and added some of ethanol & and kept overnight with occasional shaking and then filtered of ethanol the extract and concentrated it to half the volume over a water bath.

Stationary phase: - the pre-coated plates with silica gel 60F 254 of 0.2 mm thickness.

Mobile phase:- ethyl acetate: methanol (8:2), Toluene: ethyl acetate:methanol:water(6:2.5:1:0.5).

Volume of test sample applied: - 8 u

Spray reagent: - 5% methanolic H_2So_4

IV. RESULT AND DISCUSSION

The analytical results of parameters i.e. moisture content, water soluble extractive value, alcohol soluble extractive value, total ash, pH, phytochemical tests i.e. alkaloid, tannin, resin, saponin, flavonoids, carbohydrate, protein and HPTLC test are given below.

Table I. Moisture Content

S.No.	Perti. + 5g. Pow. Wt. (g.)	Wt. After 6 hrs. (g.)	Wt. After 6.30 hrs. (g.)	Difference (g.)
1.	20.8713	20.5278	20.5309	0.3404
2.	19.9328	19.5936	19.5961	0.3367
3.	19.0512	18.7121	18.7143	0.3369

Avg. = 0.338g.
Total moisture content = 0.338x100/5= 6.76g.

Table II. Total Ash

S.No.	Crucible wt. (g.)	I Reading (g.)	II Reading (g.)	III Reading (g.)	Difference (g.)
1.	36.2115	34.4958	34.3735	34.3662	0.1547
2.	39.1845	37.4964	37.3417	37.3362	0.1517
3.	38.5391	36.8138	36.6939	36.6905	0.1514
4.	36.3802	34.5526	34.5328	34.5318	0.1516
5.	36.0829	34.3910	34.2425	34.2346	0.1517
6.	40.5358	38.8295	38.7038	38.6917	0.1559

Avg. = 0.15283 g.
Total Ash value = 0.15283x100/2=7.641g.
Where difference = III reading – Crucible Wt.

Table III. Acid Insoluble Ash Value

S. No.	Initial Rading (g.)	Post Reading (g.)	Difference (g.)
1.	34.5934	34.2844	0.309
2.	37.6198	37.2815	0.3383
3.	36.9618	36.6383	0.3235

Avg. = 0.3235g.
Percentage Value of Acid insoluble Ash = 0.3236x100/2=16.18%

Table IV. Water Soluble Ash Value

S. No.	Initial Rading (g.)	Post Reading (g.)	Difference (g.)
1.	34.8081	34.4891	0.319
2.	34.4369	34.2032	0.2337
3.	38.9538	38.6571	0.2967

Avg. = 0.28313 g.
Percentage value of water insoluble Ash= 0.28313x100/2=14.156%

Table V. Alcohol Soluble Extractive (ASE)

S.No.	Pre wt. of petri (g.)	Post wt.	Difference (g)
1.	37.4705	37.4989	0.0284
2.	43.7685	43.7975	0.029
3.	41.7696	41.8013	0.0317

Avg. = 0.0297
Percentage Value of ASE = 0.0297x500=14.85%

Where 2gm. sample in 100 ml. Also. In each flask.

Table VI. Water Soluble Extractive(WSE)

S.No.	Pre wt. of petri (g.)	Post wt.	Difference (g)
1.	36.3865	36.4082	0.0217
2.	40.4270	40.4507	0.0237
3.	47.6261	47.6492	0.0231

Avg. = 0.0228

Percentage Value of WSE = 0.0228x500=11.416%

Where 2g. sample in 100 ml. water. In each flask

vii- P^H Value : 5.32

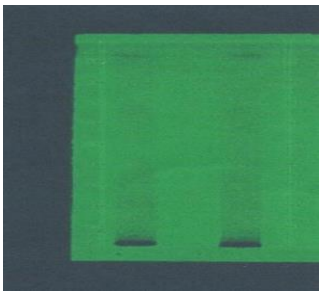
viii- HPTLC :

Toluene : Ethyl Acetate

7 : 3

- R_f Value of Nimba at 254 nm. before dervatization

S.No.	R_f of Spot A	R_f of Spot B	Colour
1.	0.21	0.21	Black
2.	0.93	0.93	Black

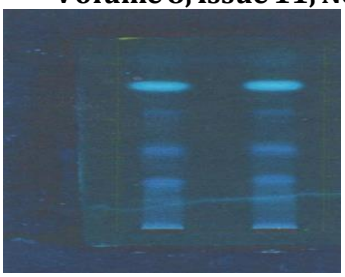


- R_f Value of Nimba at 366 nm. before dervatization.

S.No.	R_f of Spot A	R_f of Spot B	Colour
1.	0.45	0.45	Blue
2.	0.61	0.61	Blue
3.	0.85	0.85	Flourecent

- R_f Value of Nimba at 254 nm. before dervatization- *Nil*
- R_f Value of Nimba at 366 nm. before dervatization

S.No.	R_f of Spot A	R_f of Spot B	Colour
1.	0.11	0.11	Blue
2.	0.26	0.26	Blue
3.	0.44	0.44	Blue
4.	0.63	0.63	Brown
5.	0.80	0.80	Blue
6.	0.87	0.87	Blue
7.	0.96	0.96	Light Green



- Visible Light after derivatization

S.No.	R_f of Spot A	R_f of Spot B	Colour
1.	0.09	0.09	Brown
2.	0.63	0.63	Brown
3.	0.97	0.97	Brown

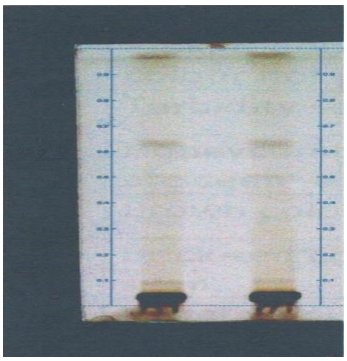


Table VII. Phytochemical analysis of parameter

S.No.	Constituents	Observation	Results
1.	Alkaloids (Wenner's test)	Yellow ppt seen	Absent
2.	Flavonoids	Pink, reddish or brown colour not produced	Absent
3.	Resins	Turbidity seen	Absent
4.	Saponins	Honeycomb like fourth are seen	Present
5.	Tannins	Brown colour not seen	Present
6.	Carbohydrates (Fehling's test)	Brick-red colour are seen	Present
7.	Protiens (Biurate test)	Violet colour obtained	Absent

p^H value : The p^H of 10% aqueous solution of drug was found to be 5.3.

The results of study showed that moisture content and total ash value were found 6.76 g. and 7.641g. respectively. The value of acid insoluble ash and water soluble ash were found 16.18% and 14.156 % respectively. The percentage of alcohol soluble extractive value and water soluble extractive values were found 14.85 % and 11.416 % respectively.The phytochemical parameters i.e. Saponin, Tanin, Carbohydrate were also present in the samples.

V. CONCLUSION

The above study revealed that the drug was authentic and genuine. The studied parameters can be used as diagnostic tools for identification of drug. Neem leaves should be utilized as remedial i.e. antiseptic and anti-microbial purpose. The neem may be used as an interal medicine to lower blood glucose level, treat peptic ulcers and kill intestinal parasites. Further pharmacological studies can lead the new drug discovery.

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