Preliminary Phytochemical Analysis, Antimicrobial and Antifungal Activities of Seed Extract from *Guazuma tomentosa Kunth.*

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ABSTRACT:-

Guazuma tomentosa is one of very important medicinal plant. It is also known as *Guazuma umbifolia* (commonly known as Mutamba or Bhadraksha or Guacimo) belonging to family Sterculiaceae. Traditionally whole plant is used for its multipurpose benefits, e.g. As astringent, in cold, in cough, in diarrhea, as diuretic, in dysentery, in venereal diseases etc.

Its non-medicinal uses involves, as a fuel wood, in making of charcoal, ropes (bark and stem because of their tough and fibrous nature). The present study deals with the preliminary phytochemical screening reveals the presence of alkaloids, carbohydrates, protein and amino acid, flavonoids, tannins, phenols, fixed oils, glycosides and saponins. Five crude extracts were prepared from the seeds (fruits) *of Guazuma tomentosa* using a different solvent by Soxhlet method. The extracts were subjected to screening to detect potential antimicrobial activity against *E.coli, Staphylococcus aureus, Pseudomonas syringae, Aspergillus niger, Aspergillus flavous,* as standard by agar well diffusion method. The aim of our present study was to find out the preliminary phytochemical screening and antimicrobial potential of different extracts of seed of *Guazuma tomentosa*.

The different extracts such as Ethanol, Methanol, Petroleum ether, n-Hexane and aqueous extracts exhibit comparable antimicrobial activity with the control.

Key words: - Gauzuma tomentosa, Gaucimo, Mutamba, Saponins, Diuretic, Venereal

Introduction:-

Taxonomical classification :-

Kingdom - Plantae

Division - Magnoliophyta

Class - Magnoliopsida

Order - Malvales

Family - Sterculiaceae

Genus - *Guazuma*

Species - *Guazuma tomentosa*

Herbal medicines were used to cure human ailments in every possible condition. Majority of population of developing country still rely on herbal medicines for primary health care. Medicinal herbs are moving from fringe to mainstream use with a great number of people

seeking remedies and health approaches free from seeking side effects caused by synthetic chemicals (Dubey N.K.et al. 2002).Worldwide over 80 % of the people depend on medicinal plant species to meet their day today health care (WHO, Geneva, Switzerland 2002). Medicinal plants used as sources for extracts or pure products for therapeutic use represent a rapidly expanding area of health science (Chopra R.N et al 1956). Higher plants, as sources of medicinal compounds have continued to play a dominant role in the maintenance of human health since ancient times. It is reported that over 50 % off all modern clinical drugs are of natural product origin and natural products play an important role in drug development programs in Pharmaceutical industry (Onorato, MMJ. Borucki.G.Baillargeon,D.P.Paar1999).*Guazuma tomentosa*is a plant native to tropical America, Equador and Colombia. Despite of its etno-pharmocological uses, presently it is proven to have many therapeutic valuable uses because of the presence of many phyto-constituents e.g. colistin, colatannins, catechins, caffeine, kaempferol, procyanidin B-2, procyanidin B-5, procyanidin C-1, tartaric acid, theobromine, xanthan gum, etc. The proven pharmacological activities involve, anti-diabetic, anti-hypertensive, anti-microbial, anti-oxidant, anti-ulcer, neurological, anti-secretory, cytotoxic, uterine stimulating activity and as a hair growth promoter.

Materials and Method :-

Materials

a) Nutrient agar medium
b) PDA medium

Apparatus

a) Sox-late apparatus
b) Autoclave
c) Refrigerator
d) Laminar air flow
e) Water bath
f) Microscope

Microorganism

Bacterial strain

a) Staphylococcus aureus
b) Escherilia coli
c)Psuedomona syringae

Fungal Strain

a) Aspergillus niger
b)Aspergillus flavous

Method:-

A) Collection, identification and processing of plant material :

Dried (ripen) seeds were collected from local Botanical garden, H.P.T Arts & R.Y.K Science College Campus and Pandit Neharu Botanical Garden, Nashik. Plant was correctly identified with the help of Flora of Maharashtra and Flora of Nashik district. Plant material was then crushed to fine powder with electric blender and stored in airtight bottles. This sample was used for extraction of organic compound.

Extraction of organic crude material from seed of *Guazuma tomentosa* :

50 gm of seed powder sample weighted and used for soxlation.

Solvent used:

Depending on polarity the following solvent selected

1. Ethanol 2.Methanol 3.Petroleum ether 4.Hexane 5.Distilled water

a) Phytochemical analysis of plant extract:

The phytochemical are essential to metabolism and chemical process of plant body. The phytochemical are studied alkaloids, terpenoids, steroids, flavonoids, glycosides, tannins and saponin.

IDENTIFICATION TEST :

The test were done to find the presence of active chemical such as alkaloids, glycosides, terpenoids, steroids, flavonoids, saponin, tannin by the following procedure.

Test for Alkaloids (Evans2002):

Solvent free extract 50 gm is stirred with ml of dilute hydrochloric acid and filtered. The filter is tested carefully with various alkaloid reagents as follows.

a. Mayer's test: To a few ml of filtrate, a drop or two of Mayer's reagent are added by the side of test tube. A white or creamy precipitate indicates the test as positive.

Mayer's Reagent: Mercuric chloride (1.358 gm) is dissolved in 60 ml of water and potassium iodide (5.0 gm) is dissolved in 10 ml of water. The two solutions are mixed and up to 100ml with water.

b. Wagner's test (Wagner 2004): To a few ml of filtrate, few drops of Wagner's reagent are added by the side of the test tube. A reddish brown precipitate confirms the test as positive.

Detection of Carbohydrates and Glycosides: The extract (100 gm) is dissolved in 5 ml of water and filtered. The filter is subjected to the following tests.

a.Mollich's test: 2 ml of filtered, two drops of alcoholic solution of a napthol are added, the mixture is shaken well and 1 ml of concentrated sulphuric acid is added slowly along the sides of the test tube and allowed to stand. A violet ring indicates the presence of carbohydrates.

b. Barfoed's test: To 1 ml of filtered, 1 ml of Barfoed's reagent is added and heated on a boiling water bath for 2 min. red precipitate indicates presence of sugar.

Barfoed's reagent: Copper acetate, 30.5 gm is dissolved in 1.8 ml of glacial acid.

c. Benedict's test: To 0.5 ml of filtrate, 0.5 ml Benedict's reagent is added. The mixture is heated on a boiling water bath for 2 min. A characteristic colored precipitate indicates the presence of sugar.

d. To 3 ml of the aqueous extract was added about 1 ml of iodine solution. A purple color at the interphase indicates the presence of carbohydrates.

e. Keller Kiliani test :2 ml of extract was dissolved in 2 ml of glacial acetic acid containing one drop of ferric chloride solution. The mixture was then poured into the test tube containing 1 ml of concentrated sulphuric acid. A brown ring at the interphase indicates the presence of deoxe sugar characteristics of cardenolides.

Detection of Saponin: The extract (50mg) is diluted with distilled water and up to 20 ml. The suspension is shaken in a graduated cylinder for 15 min. A two cm layer of form indicates the presence of saponin.

Detection of Proteins and Amino acids : The extract (100 mg) is filter paper and the filtrate is subjected to tests for proteins and amino acid.

a. Million's test: 2 ml of filtrate, few drops of Million's reagent are added. A white precipitate indicates the presence of proteins.

b. Ninhydrin test: Two drops of ninhydrin solution (10 mg of ninhydrin in 200 ml of ethanol) are added to 2 ml of aqueous solution filtrate. A characteristic purple color indicates the presence of amino acids.

Detection of phenolic compounds and Tannins:

Ferric chloride test:

a) The extract (50 mg) is dissolved in 10 ml of distilled water. To this, few drops of neutral 5% ferric chloride solution are added. A dark green color indicates the presence of phenolic compounds.

Detection of Gum and Mucilage: The extract (100mg) is dissolved in 10 ml of distilled water and to this 25 ml of absolute alcohol is added with constant stirring. White or cloudy precipitate indicates the presence of gums and mucilage.

Glycoside: Glycosides are compounds which upon hydrolysis give rise to one more sugars (glycones) and a compound which is not a sugar (aglycone or genine). To the solution of the extract in glacial acetic acid, few drops of ferric chloride and concentrated sulphuric acid and observed for a reddish brown coloration at the junction of two layers and the bluish green color in the upper layer.

Terpenoids and Steroids: 0.4 mg of extract was treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated solution of sulphuric acid was added slowly and red violet color for steroids (Siddique and Ali 1997)

Flavonoids: 0.4 ml of extract solution was treated with 1.5 ml of 50 % methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated hydrochloric acid was added and red color for flavones

Tannins: To 0.5 ml of extract solution 1 ml of water 1-2 drops of ferric chloride solution was added. Blue color was observed for Gallic tannins and green black for catecholic tannins.

Fixed oils and Fats: A small quantity if extract is pressed between two filter papers. Oil stain on the paper indicates the presence of fixed oils.

Saponification test:
A few drops of 0.5 N alcoholic potassium hydroxide solutions are added to a small quantity of extract along a drop of phenolphthalein. The mixture is heated on water bath for two hours. Formation of soap partial neutralization of alkali indicates the presence of fixed oils and fats.

C) Anti microbial Activity:-

Inoculums: The microorganism isolated and incubated at 35 $\pm$ 2˚C. For 4 hours. The turbidity of the resulting bacterial adjusted to turbidity of the resulting bacterial adjusted to turbidity equivalent to 1 McFarland turbidity is equivalent to approximately 3.0 X 108 CFU/ml.

Bacterial and fungal strain used:-

To study antimicrobial activity following three bacterial strains and two fungal strains used.

1. *Escherichia coli*
2. *Aspergillus niger*
3. *Aspergillus flavous*
4. *Staphylococcus aureus*
5. *Pseudomona syringae*

Agar well diffusion method:-

The modified agar well diffusion method was employed Muller-Hinton agar plates were inoculated by streaking the swab over the entire sterile over the entire sterile agar surface. This procedure was repeated striking two more times, rotating the plate approximately 60˚ each time to ensure even distribution of the inoculums. As a final step the rim of the agar was also swabbed. After allowing the inoculums to dry at room temperature, 6-mm diameter wells were bored in the agar.

Each extract was check for antimicrobial activity by introducing 100 µl of 4000 µg/ml concentration into triplicate well simultaneously and the dilution medium for the positive control was respective solvents. The plates were allowed to stand at room temperature one hour for extract to diffuse into the agar and then they were incubated at 35± 2˚ C for 24 hour. Solvents extract were showed area of inhibition that solvent extract further analyzed for find out minimum inhibition concentration (MIC) by using 25 µ ml , 50 µl,75 µl, 100µl and against positive control used pure solvent 100 µl. The plates were allowed to

stand at room for 1 hour for extract to diffuse into the agar and then they were incubated at 35± 2˚ C for 24 hour zone of inhibition measured with scale and observations were noted in notebook.

Table No 1 – Physicochemical characteristics of different extracts from seed of *Guazuma tomentosa*

Solvent	Initial weight of powder (gm)	Final weight of powder (gm)	Weight of crude extract (gm)	Color of extracts
Distilled water	50	47.50	2.50	Coffee
Ethanol	50	48.00	2.00	Dark brown
Methanol	50	49.00	2.40	Brown
Petro. ether	50	48.00	2.20	Light green
Hexane	50	48.85	2.50	Pale yellow

Table No 2:- Preliminary phytochemical analysis of crude extract from seed of *Guazuma tomentosa*.

Sr.No.	Phytochemical test	Ethanol	Methanol	Petro.ether	Hexane	D.W
1.	Alakaloid					
	a.Mayer's test	++	+++	+	-	++
	b.Wagner's test	++	++	+	+	-
2.	Carbohydrate					
	a.Mollich's test	+	-	++	+	-
	b.Barfoed's test	-	-	-	-	-
	c.Benedict's test	-	-	-	-	-
	d.Keller-Kiliani test	-	-	+	++	-
3.	Saponins					
	a.Foam test	-	-	++	+	-
4.	Proteins and Amino acids					
	a.Million's test	++	++	-	-	+++
	b.Ninhydrin test	-	-	++	-	+++
5.	Phenolic compounds					
	a.Ferric test	-	-	+	+	-
6.	Tannins					
	a.Gelatin test	++	++	+	+	+++
7.	Gum and Mucilage					
	a.95% alcohol	-	-	++	++	-
8.	Fixed oils and Fats					
	a.Spot test	++	++	+	+	+++
	b.Saponification test	++	++	+	+	-
9.	Carbohydrate					
	a.Iodine test	-	-	-	-	++
10.	Flavonoid	+++	++	+	+	++
11.	Glycosides	-	-	++	++	-

MEDIA PREPARATION:-

PDA Medium (Potato Dextrose Medium) (For Fungi)

1. Potato- 200gm
2. Dextrose- 20gm
3. Agar- 15gms
4. Distilled water - 1000ml

Nutrient Agar Medium (for Bacteria)

1. Yeast extract- 10gm
2. NaCl - 5gm

3. Peptone- 10gm
4. Distilled Water- 1000ml
5. Agar- 20gm

Table No.3 Antimicrobial Activity of *Guazuma tomentosa* seed

Sr.No.	Microorganism strain	Extract					
			Zone of inhibition in cm				Control
1.	*E.coli*	Conc. of extract in µl	25	50	75	100	
		Ethanol	0.3	0.6	0.9	1.3	Nil
		Methanol	0.4	0.8	1.1	1.5	Nil
		Petro.ether	0.3	0.5	0.7	1.2	Nil
		Hexane	Nil	Nil	Nil	Nil	Nil
		D.W	Nil	Nil	Nil	Nil	Nil
2.	*S.*aureus						
		Ethanol	0.2	0.5	0.6	0.8	Nil
		Methanol	Nil	Nil	Nil	Nil	Nil
		Petro.ether	0.3	0.4	0.6	0.9	Nil
		Hexane	Nil	Nil	Nil	Nil	Nil
		D.W	Nil	Nil	0.3	0.5	Nil
3.	*A.niger*						
		Ethanol	0.2	0.5	0.7	0.8	Nil
		Methanol	0.3	0.4	0.7	0.9	Nil
		Petro.ether	Nil	Nil	Nil	Nil	Nil
		Hexane	0.4	0.7	0.8	1.1	Nil
		D.W	Nil	Nil	0.3	0.5	Nil
4.	*A.flavous*						
		Ethanol	0.3	0.5	0.6	0.9	Nil
		Metahnol	0.2	0.3	0.5	0.7	Nil
		Petro.ether	Nil	Nil	Nil	Nil	Nil
		Hexane	Nil	Nil	Nil	Nil	Nil
		D.W	0.2	0.3	0.5	0.7	Nil
5.	*P.syringae*						
		Ethanol	0.1	0.3	0.3	0.5	Nil
		Methanol	Nil	Nil	Nil	Nil	Nil
		Petro.ether	0.2	0.2	0.4	0.5	Nil
		Hexane	Nil	Nil	Nil	Nil	Nil
		D.W	Nil	Nil	Nil	Nil	Nil

Results and Discussion:-

The plant material was subjected to successive extraction with Ethanol, Methanol, Petroleum ether, Hexane and distilled water. Result of phytochemical properties is showed in (Table No. 1). Phytochemical studies of different extract reveled presence of alkaloids, carbohydrates, steroids, saponin, tannins and phenols etc. in (Table No. 02). All the plant extracts detected presence of alkaloids. Carbohydrates strongly present in Petroleum ether and Hexane extract. Saponin and phenolic compounds are only present in petroleum ether and Hexane extract. Fixed oil and volatile oils are strongly present in all extract but in Hexane it is very less. Flavonoids and Glycosides are strongly present in petroleum ether and Hexane extract. The phytochemical compounds identified in the presence study this are

bioactive and it shows various pharmacological activities as Astringent in cold, in cough, in diarrhea, as diuretic in dysentery, in venereal diseases etc.(Minakshi Sharma, Shruti Chopra, Shyam Baboo Prasad)

Guazuma tomentosa plant seed five extract (Ethanol, Methanol, Petroleum ether, Hexane and Distilled Water) tested against human pathogenic bacteria and fungi (Table No. 03). Out of this five extracts, Ethanol and Petroleum ether, showed high in vitro antibacterial activity against S. aureus. Methanol extract showed strong antibacterial activity against *E.coli*, *P.syringae*, *A. flavous* and *A.niger* fungal pathogen strongly inhibited by Hexane and Petroleumether extract. Ethanol and Petroleum ether showed antimicrobial activity against bacteria *P. syringae*. Pure solvent used as control not showed any activity against microorganism. Further studies carried out isolation and purification of medicinally important compounds is useful for various pharmacological activities and it is also help curing the various diseases.

CONCLUSION:-

The extract made in Ethanol, Methanol, Petroleum ether, Hexane and Distilled water has presence of different secondary metabolites. The phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, saponins and glycoside with a very high content in petroleum ether.

The sequentially extracted Ethanol, Methanol, Petroleum ether, Hexane and Distilled water extracts were further taken for antimicrobial and antifungal activities and were tested at 25, 50, 75 & 100 µl for each of the extract with reference to pure solvent as control.
The organisms were taken, three bacterial and two fungal *strains E. coli, S. aureus,P. syringae A. niger, A. flavous*, Antimicrobial activity observed against Ethanol but Hexane extract has not maximum impact in inhibition. The result of this study validates the use of Ethanol extract of this species in ethanomedicine providing lead for antifungal and antimicrobial for above strain.

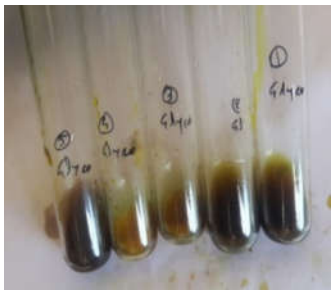
SUMMARY:-

The use of medicinal plants as therapeutic agents in herbal medicines was used to cure human aliments in every possible condition. *Guazuma tomentosa* is one of very important medicinal plant, the ethno pharmacological utilization of plant were used in the treatment of vast array of diseases and patho physiological disorders ranging from anti dysenteric styptic to sudorific. More or less all of the parts of tree, containing diverse chemical constituents were used in the treatment Of Alopecia, Bruises, Cough, Hemorrhage infection, Leprosy, Nephritis, Asthma, Childbirth, Dematosis, Hemorrhoids Influenza, Liver problem, Pneumonia, Bronchitis, Constipation, Grippe, Hypertension, Kidney problem, Malaria, Prostate problem, Skin problems, Inflations, Stomach troubles, Stomachache, Wound And Uterine Pain, Diarrhea, Astringent etc.

Photographs of Phytochemical Identification test:-

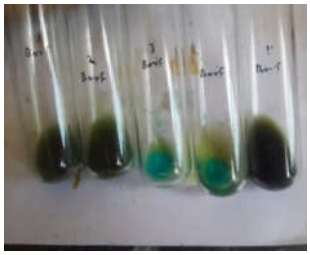
Mollich's test

Flavonoid test

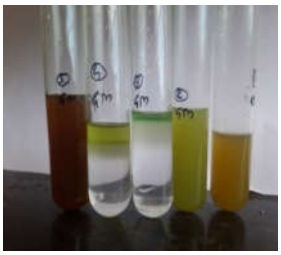


Glycosides test

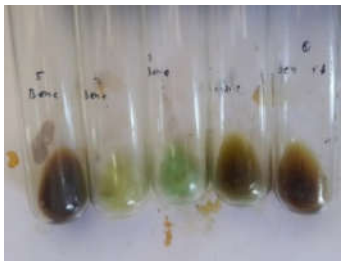
Tannin test



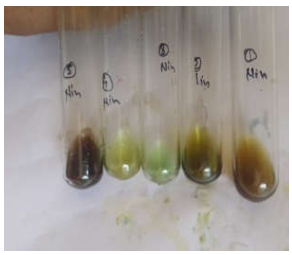
Barfoed's test



Gum and Mucilage test



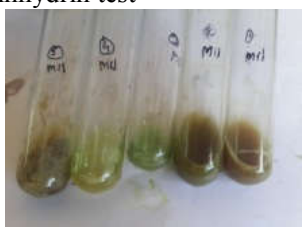
Benedict's test



Ninhydrin test

Ferric Choride test

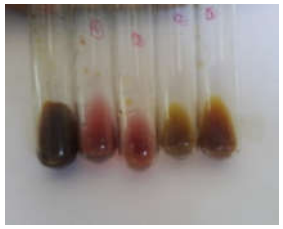
Streroid test



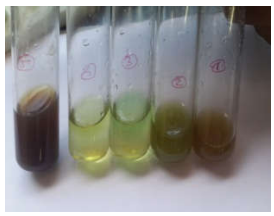
Million's test

Keller-Killani test

Iodine test



Mayer's test



Wagner's test

Oil & Fat test for Methanol

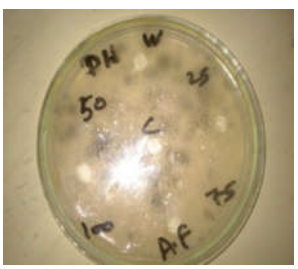
Oil & Fat test for Petro.ether

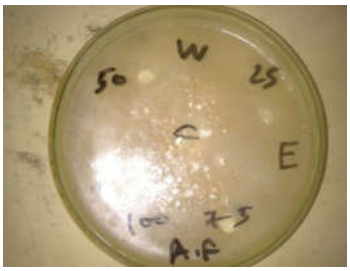
Oil & Fat test for Hexane

Oil & Fat test for Ethanol

Oil & Fat test for Distilled Water

Photographs of petri plates showing Antimicrobial and Antifungal zone of inhibition :-





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