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Full Length Research Paper

Comparative pharmacognostical and antimicrobial studies of *acacia* species (Mimosaceae)

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Various *Acacia* species have been reported to be effective against a variety of disease including malaria, leprosy and most concerning cancer. The fresh plant parts of different *Acacia* species are considered as astringent, spasmolytic, demulcent, anthelmintic and abortifacient in Indian traditional medicine system. Currently, numerous herbal products derived from *Acacia* species are available in market. In present exploration, a total of five species of genus *Acacia* including: *Acacia nilotica* ssp. *indica* (Benth.) A. F. Hill, *Acacia tortilis* (Forsk.) Hayne, *Acacia senegal* (L.) Willd., *Acacia catechu* (L.)Willd, *Acacia jacquemontii* Benth were undertaken for preliminary ethnomedicinal and antimicrobial screening. Subsequently, the two most active species: *A. catechu* and *A. nilotica* were further considered for detail pharmacognostical studies. During antimicrobial screening experiments, *A. catechu* and *A. nilotica* exhibited highest activity against three bacterial (*Escherichia coli, Staphylococcus aureus* and *Salmonella typhi*) and two fungal strain (*Candida albicans* and *Aspergillus niger*). The pharmacognostical study revealed that both species (*A. catechu* and *A. nilotica*) can be distinguished on the basis of their macroscopic, microscopic and phytochemical characters. Different plant parts (bark and pods) of both species were found to contain various secondary metabolites such as alkaloids, flavanoids, tannins and sponins.

Key words: Antimicrobial activity, pharmacognosy, abortifacient, antiplatelet aggregatory, saponins.

INTRODUCTION

Acacia is the most significant genus of family: Leguminosae, first of all described by Linnaeus in 1773. It is estimated that there are roughly 1380 species of *Acacia* worldwide, about two-third of them native to Australia and rest of spread around tropical and subtropical regions of the world (Maslin et al., 2003; Orchard and Maslin, 2003).Gamble, (1918) have reported more than 40 species of this genus in India in his 'Flora of Madras Presidency.' *Acacia* species are commonly known as 'Babool' in India and ethnomedicinally have long been used for the treatment of skin, sexual, stomach and tooth problems. Still many herbal products derived from *Acacia species* are sold in market in their pure or mixed form such as: Babool tooth pest, Ayur Shampoo, Nyle Shampoo etc.

The scientific efforts in field of pharmacognosy and pharmacology during last fifty years have revealed that many species of this genus have been reported to be used against a variety of disease. *Acacia nilotica* has been proved as effective medicine in treatment of malaria, sore throat (aerial part) and toothache (bark) (Chopra et al., 1956; Shetty, 1977; Jain, 1991; Joshi, 1994; Jain, 1997; Jain et al., 2005; Kubmarawa et al*.,* 2007). Choudhary et al. (1984) have tested the anti-fertility activity of *A. nilotica* pods and nuts. The methanolic extracts of *A. nilotica* pods have been claimed against HIV-PR (Hussein et al., 1999; Bessong and Obi, 2006). The antiplatelet aggregatory activity of this species was reported in animal model by Shah et a1. (1997), that was possibly due to blockade of calcium influx through membrane calcium channels on target cell. Currently, one group of researchers has tested the antiplasmodial activity of *A. nilotica* ethyl acetate extract against different chloroquine resistant and sensitive strains of *Plasmodium falciparum* (El- Tahir et al*.,* 1999). The fresh plant parts of this species have been reported to be most active against Hepatitis C virus by Hussein et al., (2000).

Acacia catechu (Figure 1-10) showed an anti-cell adhesive activity at non-cytotoxic concentration, that was

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Abbreviations: E, Epidermis; C, Cortex; X, Xylem; Sx, Secondary xylem; Px, primary xylem; Ph, Phloem; Sg, Starch granules; Pr, Pericycle; Cb, Cambium; Vc, Vascular cylinder; Mr, medullary rays.

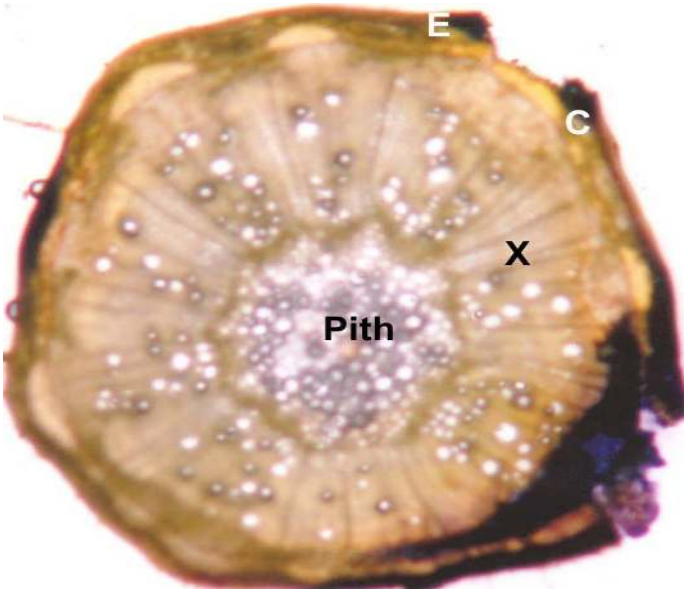

Figure 1. Microphotograph of a cross section of stem of *Acacia catechu* showing colour pattern of histochemical localization of cellulose (Light Grayish).

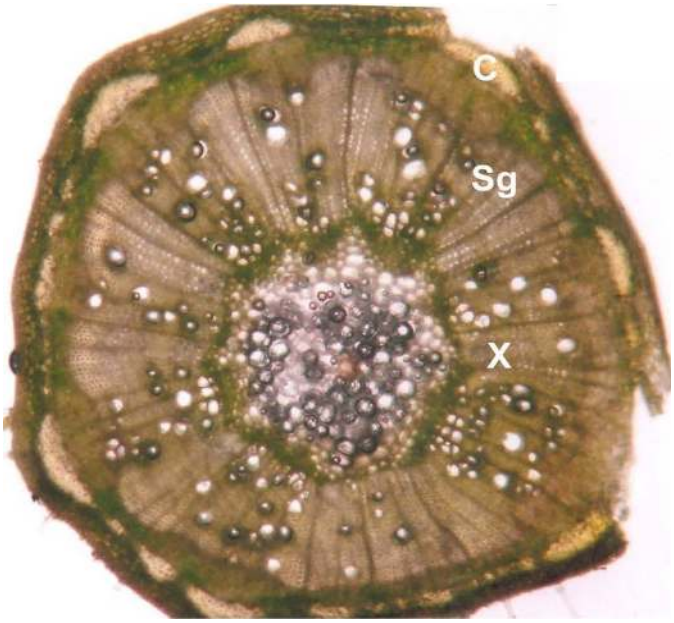

Figure 3. Microphotograph of a cross section of stem of *Acacia catechu* showing colour pattern of histochemical localization of starch (Dark Gray).

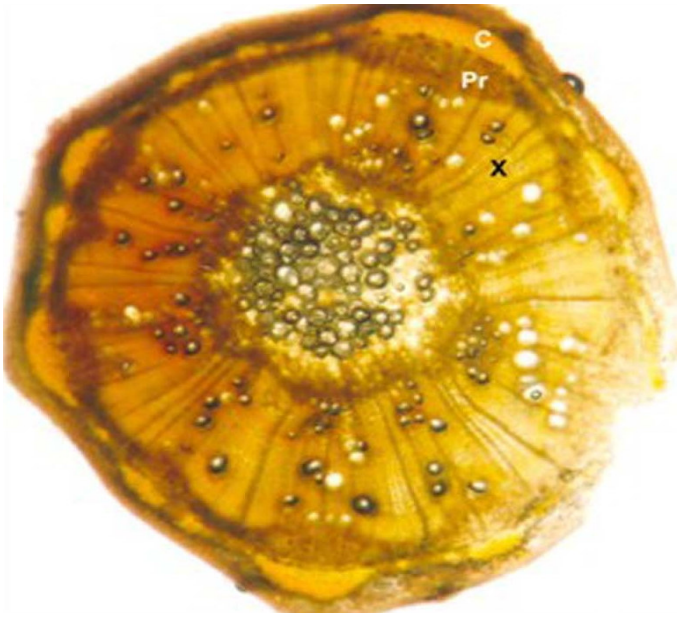

Figure 2. Microphotograph of a cross section of stem of *Acacia catechu* showing colour pattern of histochemical localization of tannins (yellow-Brown).

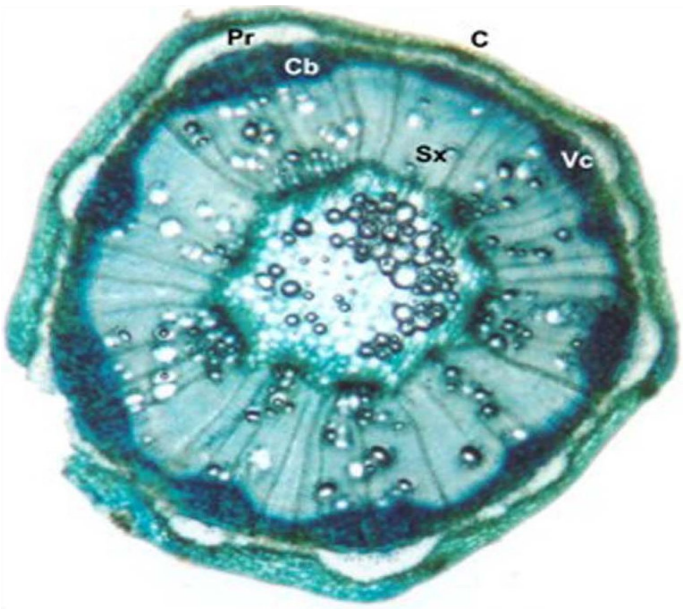

Figure 4. Microphotograph of a cross section of stem of *Acacia catechu* showing colour pattern of histochemical localization of proteins (Green).

possibly due to saikosaponins, with a beta-hydroxy group at C-16 position, isolated from bark of *A. catechu* plant. The seeds of this plant have also showed leukoagglutinating activity against whole leucocytes and mononuclear cells from the patients with chronic myloid leukemia (Agrawal and Agrawal, 1990).

Throughout literature survey, it was found that very little or no work on pharmacognostical and antimicrobial activity of *Acacia* species, considered in this investigation on record. In present investigation, a total of five species of genus *Acacia* including: *A. nilotica* ssp. *indica* (Benth.) A. F. Hill, *Acacia tortilis* (Forsk.) Hayne, *Acacia senegal* (L.) Willd., *A. catechu* (L.) Willd, *Acacia jacquemontii* Benth were undertaken for preliminary ethnomedicinal and antimicrobial screening. Subsequently two most active

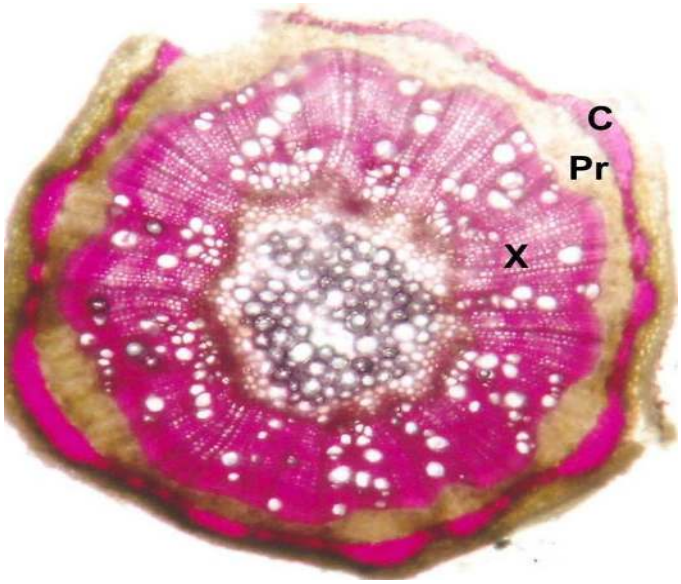

Figure 5. Microphotograph of a cross section of stem of *Acacia catechu* showing colour pattern of histochemical localization of lignin (Red-Pink).

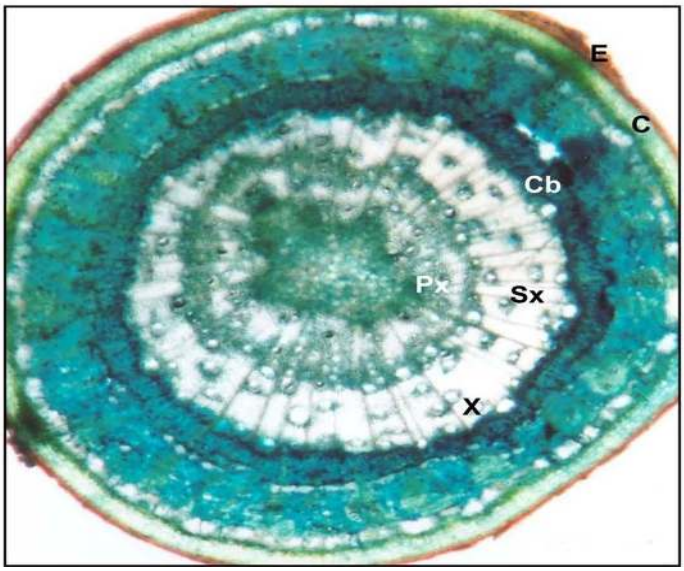

Figure 6. Microphotograph of a cross section of stem of *Acacia nilotica* showing colour pattern of histochemical localization of proteins (Green).

species; *A. catechu* (L.)Willd and *A. nilotica* were further considered for detail pharmacognostical studies.

MATERIAL AND METHODS

Ethnomedicinal study

Ethnomedicinal survey of selected *Acacia* species were carried out during Oct. 2005 to Sept. 2006 in Shekhawati areas of Rajasthan, India, including three districts: Churu, Siker, Jhunjhunu.

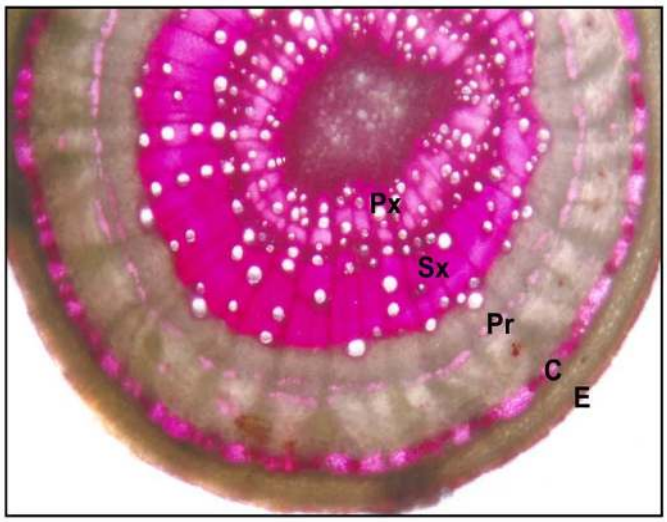

Figure 7. Microphotograph of a cross section of stem of *Acacia nilotica* showing colour pattern of histochemical localization of lignin (Red-Pink).

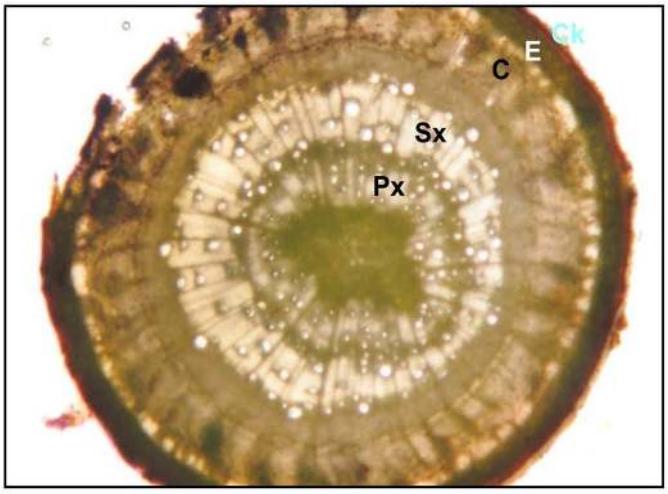

Figure 8. Microphotograph of a cross section of stem of *Acacia nilotica* showing colour pattern of histochemical localization of cellulose (Grayish).

Geographically, this is a part of Thar Desert, situated between 27 - 29 N latitude to 74 - 76 E longitude. The interviews were conducted with local Ayurvedic vaidyas, tribal peoples and knowledgeable individuals, ranging in age between 35 and 70 years old. All the information regarding plant species, biological forms, habitat, local names and uses were documented. A voucher specimen of each studied plant species has been deposited in Herbarium, Department of Botany, University of Rajasthan.

Antimicrobial screening

The fresh plant parts of five *Acacia* species were collected and air dried at room temperature for 2 weeks, with no direct sunlight. Dried part then stored at -20°C before using them individually to solvent extraction procedures. The antimicrobial activity of selected plant

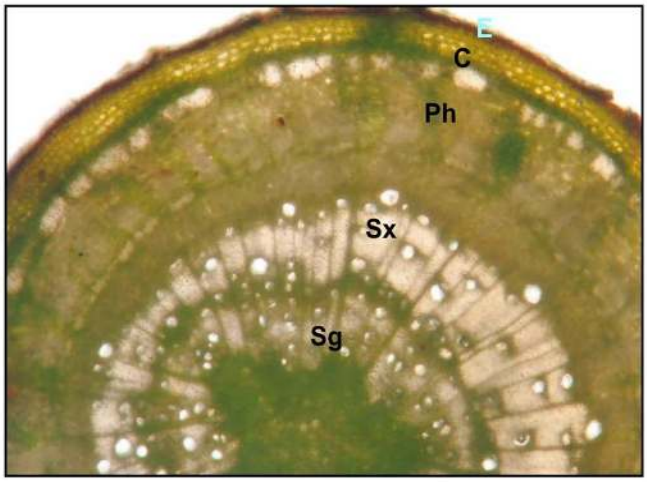

Figure 9. Microphotograph of a cross section of stem of *Acacia nilotica* showing colour pattern of histochemical localization of starch (Dark Gray).

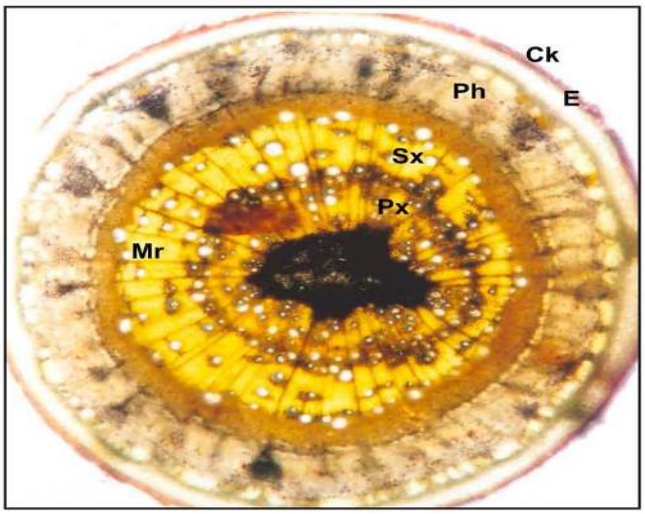

Figure 10. Microphotograph of a cross section of stem of *Acacia catechu* showing colour pattern of histochemical localization of tannins (yellow-Brown).

parts were tested against different bacterial and fungal strains which were obtained from SMS hospital and Department of Biotechnology, MGIAS, Jaipur. The microbial strains with their laboratory registration number were: *Bacellus cereus* (SB-625), *Escherichia coli* (SB-981), *Pseudomonas aeroginosa* (SB-845), *Salmonella typhi (*SB-1014), *Staphylococcus aureus (*MB-123), Candida *albicans* (MF-098), *Aspergillus niger* (MF-023), *Microsporum canis* (SF-472).

The disc diffusion method was used to test the antimicrobial activity in present investigation. The antibacterial activity of plant extract was tested on Mueller Hinton Agar (MHA) plates and antifungal activity was tested on Sabouraud Agar (SA) plates. The MHA or SA plates were prepared by pouring a few ml of molten media into sterile petriplates. The plates were allowed to solidify for 5 min and appropriate amount of inoculum's suspension (0.1 %) was swabbed uniformly on agar medium. A sterile disc was placed on the surface of medium and an appropriate concentration of extracts (5 mg/disc) was loaded on it. The extract was allowed to diffuse for 5 min and the plates were kept for incubation at 37°C for 24 h. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter. Ampicillin and amphotericin (5 mg/ml each) were used as standard for antibacterial and antifungal activity testing respectively.

Pharmacognostical studies

Different macroscopic characters including height, size, arrangement, texture, and surface characters of different plant parts of both *A. catechu* (L.)Willd and *A. nilotica* were studied and tabulated. For microscopic studies, transverse sections (TS) were prepared and histochemically stained using different staining methods including: Lugol's iodine for tannin (Haridas and Kumar, 1985); IKI-H_2S0_4 method for cellulose (Johansen, 1940); Iodine-potassium iodide (I_2-KI) method for starch (Johansen, 1940); phloroglucinol HCl test for lignin (Johansen, 1940) and amido-black method for total protein (Weime, 1959).

For preliminary phytochemical studies, different parts (bark and pods) of *A. catechu* and *A. nilotica* were collected and dried under shade. These dried materials were mechanically powdered after keeping them in an oven at 35°C for 24 h. These powdered materials were further used for extraction in hexane and methanol. The qualitative identification of different phytochemicals were performed employing Mayer's reagent, Dragendorff's reagent, and Hager's reagent for alkaloids; Ferric chloride solution for tannins; Foam test for saponins; Shinoda test for flavonoids; Molisch's reagent, Fehling and Benedict's solutions for carbohydrates (Kokate,1994).

RESULTS

Ethnomedicinal study

Results of ethnomedicinal study are summarized in Table 1.

Antimicrobial screening

The results of antimicrobial activity experiments are presented in Table 2. In these experiments, the mathanolic extracts of *A. nilotica* (pods) and *A. catechu* (bark) were reported to be most active against different bacterial and fungal strains. The mathanolic extract of *A. nilotica* (pods) showed highest activity against *E. coli, S. aureus* and *A. niger,* whereas *A. catechu* exhibited its prominent activity against *S. aureus* and *C. albicans*. However, the hexane extract of *A. nilotica* was also found most active against *S. typhi*. In whole antimicrobial experiment, *Acacia Jacque-montii* was reported with weakest or no activity.

Pharmacognostical studies

Macroscopic study

The comparative macroscopic study of *A. nilotica* and *A. catechu* are compiled in Table 3.

Microscopic study

The aim of this study was to localize different plant metabolites in cell or tissues of investigated species, which is important parameter for standardization of crude drugs.

Table 1. Ethnomedicinal study of five *Acacia species*.

S.N	Name of Species	Common name	Herbarium No.	Part used	Ethnomedicinal uses
1.	*Acacia nilotica* ssp. *indica* (Benth.) A.F.Hill	Kiker	RUBL- 20248	Barks	Toothache and cough
				Leaves	Sexual weakness and diarrhea
				Seeds	Stomach complaint and skin disorder
2.	*Acacia tortilis* (Forsk.) Hayne	Israeli Babul	RUBL -20249	Bark	Skin ailments and cough
				Pods	Indigestion
				Leaves	Indigestion
3.	*Acacia senegal* (L.) Willd	Kher	RUBL- 20250	Gum	Leprosy
				Arial parts	Cough
4.	*Acacia catechu* (L.)Willd	Katha	RUBL-20281	Bark	Leucorrhoea, menstrual
				Leaves	complaints, sore mouth
				Gums	Dysentery and gonorrhoea
					Asthma
5.	*Acacia jacquemontii* Benth	Chota babul	RUBL-20282	Young branches	Toothache

Table 2. Antimicrobial screening of crude methanolic and hexane extracts of five Acacia species at 5 mg /disc in agar diffusion method measured by diameter of inhibition zone (mm).

S.N	Name of Species	Part used	Solvent extracts	ST	EC	BC	PAE	SA	CAL	MC	AGN
1	*Acacia nilotica* (Benth.) A.F.Hill	Pod	H	+++	++	+	-	-	++	-	-
			M	++	-	++	-	+++	++	-	+++
2	*Acacia tortilis* (Forsk.) Hayne	Bark	H	++	++	-	-	-	-	-	++
			M	-	++	++	-	-	-	-	++
3	*Acacia senegal* (L.) Willd	Bark	H	-	-	-	-	+++	++	-	
			M	-	++	++	-	-	++	-	++
4	*Acacia catechu* (L.)Willd	Bark	H	-	++	++	+	-	+	-	+
			M	++	++	+	+	+++	+++	-	++
5	*Acacia jacquemontii* Benth	Whole plant	H	-	-		-	-	-	-	-
			M	-	-	++	-	+	++	-	-
6	Standard (ampicillin/amphotericin)			++++	++++	++++	++++	++++	++++	++++	++++
7	Control or Blank			-	-	-	-	-	-	-	-

- : absent, +: 1-5 mm, ++ : 6-10 mm, +++ : 11-15mm, ++++ : 16 or more. H: hexane, M: methanol. ST: *Salmonella typhi,* EC: *E. coli,* BC : *Bacellus cereus,* PAE : *Pseudomonas aeroginosa,* SA: *Staphylococcus aureus,* CAL : *Candida albicans,* MC: *Microsporum canis,* AGN: *Aspergillus niger*

Table 3. Comparative macroscopic characters of two *Acacia* species

Macroscopic Characters	Acacia nilotica ssp indica (Benth.) A. F Hill	Acacia catechu (L.) Willd
1. Height	Plant 1.2-18 m tall	Plant 5- 15 m tall
2. Stem	Straight, blackish- grey	Straight and grayish brown
3. Bark	Bark is dark brown, longitudinally fissured	Bark is dark grayish brown, exfoliating in narrow strips brown and red in side.
4. Leaves	Leaf: bipinnate having 2- 11 pairs of pinnae, each of which contain 7-25 pairs of leaflets	Leaf: bipinnate having 10-30 pairs of pinnae each with 20-50 pairs of leaflets
5. Spines	Stipular, straight or may be absent	Short and hooked shaped
6. Inflorescence	Axillary pedunculate head	Axillary pedunculate spike
7.Flower	Flowers, bright yellow,scented	Flowers, Creamy whitish
8. Pods	Straight or curved and 8-12 seeded	Straight, flat brown and 3-10 seeded

Table 4. Histochemical analysis of stem of *Acacia catechu*.

S.No.	Compound	Reagents Employed	Tissue localized	Photo Plates
1.	Cellulose	Iodine-potassium iodide	Epidermis and outer cortex	Fig.1
2	Tannin	Lugol's iodine	Cortex, percicyle and xylem	Fig.2
3.	Starch	Iodine-potassium iodide	Cortex and xylem	Fig.3
4.	Protein	Amido black	Cortical, pericycle and xylem	Fig.4
5.	Lignin	Phloroglucinol	Cortex, pericycle and xylem part	Fig.5

Table 5. Histochemical analysis of stem of *Acacia nilotica*.

S.No.	Compound	Reagents employed	Tissue localized	Photo Plates
1.	Protein	Amido black	Inner cortical, pericycle, cambium and phloem	Fig.6
2.	Lignin	Phloroglucinol	Cortex, pericycle and xylem	Fig.7
3.	Cellulose	Iodine-potassium iodide	Epidermis and cortex	Fig.8
4.	Starch	Iodine-potassium iodide	Epidermis, cortex and vascular bundle	Fig.9
5.	Tannin	Lugol's iodine	Epidermis and pericycle	Fig.10

Tannin was histochemically appeared brown and localized in cortex, pericyle, xylem of *A. catechu* stem; epidermis and vascular cylinder of *A. nilotica* stem. Protein was appeared blue-greenish and localized in cortex, pericycle and xylem of *A. catechu* stem; inner cortex, pericycle, cambium and phloem of *A. nilotica* stem. Cellulose was appeared black brownish and localized in epidermis and cortex of both species. Starch granules appeared blue to black in colour and localized in cortex and xylem of *A. catechu* stem; epidermis, cortex and vascular bundle of *A. nilotica* stem. Lignin appeared dark pink and localized in cortex, pericycle and xylem part of *A. catechu* stem; Cortex, pericycle and xylem of *A. nilotica* stem. The result of whole study are summarized in Table 4 and 5.

Phytochemical study

The results of qualitative phytochemical study of *A. nilotica* and *A. catechu* are presented in Table 6. In this investigation, *A. catechu* was reported to have maximum studied compounds. Both species gave positive result for alkaloid. The mathanolic extracts of *A. catechu* bark and pods gave positive result for saponin and flavonoid.

DISCUSSION

Plant based drugs are gaining popularity because of several advantages such as fewer side effect, better patient tolerance, relatively less expensive and acceptance due to a long history of use, especially herbal medicines provide rational means for the treatment of many diseases that are incurable in other system of medicine. The aim of these investigations was pharmacognostical and antimicrobial study of selected *Acacia* species. The whole study was initiated though a preliminary ethnomedicinal survey of five *Acacia* species, conducted in many rural areas of Rajasthan. In this survey it was found that all the investigated species have a significant role in folk medicine, but *A. catechu* and *A. nilotica* are most considerable. The maximum parts of these two species were used by rural people to cure a variety of ailments *e.g. A. nilotica*: barks in toothache and cough; leaves in sexual

Table 6. Qualitative phytochemical studies of hexane and methanolic extracts of *Acacia nilotica* and *Acacia catechu* using Mayer's reagent, Dragendorff's reagent, and Hager's reagent for alkaloids; Ferric chloride solution for tannins; Foam test for saponins; Shinoda test for flavonoids; Molisch's reagent, Fehling and Benedict's solutions for carbohydrates.

	Name of compound	*Acacia nilotica* ssp *indica* (Benth.) A. F				*Acacia catechu* (L.) Willd			
		Bark		Pod		Bark		Pod	
1	Alkaloids	+	+	+	+	+	+	+	+
2	Tannins	-	+	-	-	-	+	-	+
3	Saponins	-	-	-	+	-	+	-	+
4	Flavanoids	-	+	-	+	-	+	-	+
5	Carbohydrates	-	-	+	+	-	-	+	+

- : ABSENT + : PRESENT; H: Hexane extracts; M: methanolic extracts

l weakness and diarrhea; seeds in stomach complaint and skin disorder. *A. catechu*: bark in leucorrhoea and menstrual complaints; leaves in dysentery and gonorrhoea; gum in asthma. The ethnomedicinal uses of above species have also been described by other researchers such as: *A. nilotica* (L.) Del. used in digestive system disorder, syphilis, cholera, dysentery and leprosy; *A. catechu* used in bronchitis, pain in chest, asthma and cancerous sores (Chopra et al., 1956; Gupta, 1970; Jain and Tarafder, 1970; Joshi, 1982; Das et al., 1983; Katewa et al., 2004).

The antifungal and antibacterial activity experiments confirmed that the methanolic extracts of tested plant species were more active than hexane extracts. It was found that methanolic extracts of *A. nilotica* and *A. cacia catechu* showed maximum activity whereas *A. Jacquemontii* showed weakest or no activity. This may be due to hydrophilic compounds such as polyphenols, gums (polysaccharides) and tannins, present in one or more part of investigated plant species. These compounds can be easily extracted in polar solvents such as methanol, ethanol and ethyl-acetate (Cos et al., 2006). However, the hexane extracts of these species also showed significant activity as well as methanolic extracts. This is suggestive of presence of more amounts of hydrophobic compounds in one or more part of investigated species. The anti-infective potential of plants is mainly due to a variety of antimicrobial compounds that are produced by the plants for their own purpose. These are either produced in the plant or induced after infection, these can also be induced by abiotic factors such as UV irradiation, and they have been defined as 'antibiotics formed in plants via a metabolic sequence induced either biotically or in response to chemical or environmental factors.

The antibacterial activity of *A. nilotica* gum was also assessed by one group using fresh isolated reference strain of *Actinobacillus actinomycetemcomitans, Capnocytophaga spp., Porphyromonas valisgingi, Prevotella intermedia, Peponema denticola.* The observed minimum inhibited these bacteria and their protease activity (Clark et al., 1993). In antimicrobial activity, to detect active substances present in plant extract; a concentration step is inhibitory concentration was 0.5-1.0% w/v that potentially critical and is based on evaporation of the solvent. It is advised to extract and evaporate at low temperature not to destroy any thermolabile constituent.

There are many specific recommendations that extremely influence the antimicrobial susceptibility testing experiments including; standard bioassay method, panel of test organism and culture media. As standard bioassay method, the agar diffusion method was used to detect the activity against different bacterial and fungal strains. However, several methods such as dilution, bio-autographic and conductimetric methods are offered but they are not equally perceptive and economically cost effective (Berghe and Vlietinck, 1991; Cole, 1994; Rios et al., 1988; Hadacek and Greger, 2000; Sawai et al., 2002). The inoculating temperature and incubation time are critical in agar diffusion method, so to enhance the detection limit of inhibition zones, inoculated system should kept at lower temperature for several hours before incubation.

In the panel of test organism, thee gram positive *(B. cereus, E. coli, S. typhi*) and two gram negative *(P. aeroginosa, S. aureus)* bacterial strains were used for antibacterial testing and three fungal strains (*C. albicans, A. niger* and *M. canis)* for antifungal testing. The selection of these microbial strains was totally based on two parameter; (i) easy growing behavior and availability (ii) their current medical needs. The wide spread drug resistance to these bacterial strains are also main hurdle that forced to discover new plant based antimicrobial against these strains.

Standardization of herbal drugs is most desirable part of plant based drugs designing that includes macroscopic, microscopic and phytochemical studies of investigated plant parts. Macroscopic characters involve size, arrangement, venation, texture, surface characters, markings and hardness of the plant materials. The microscopical studies (anatomical and histochemical) are often necessary to establish the botanical identity of commercial samples of medicinal plants, timbers, fibers etc. and may play an important part in checking adulteration and substitution. It involves longitudinal and transverse sectional views of the

of the drug.

Histochemical and phytochemical study of two most active species; *A. nilotica* and *A. catechu* were also performed in this investigation that confirmed the presence of various plant metabolites in plant tissue.

There is increasing evidence to support that the plants of genus *Acacia* are relatively high in bioactive secondary compound and are thus likely to hold promise for drug discovery. Secondary compounds in *Acacia* are important for a variety of functions, chief among these are Anti-cancer (triterpenoid and saponins), diuretic (glucosides), natriuretic (glucosides), important nutraceutical (poly-saccaride and gum) anti-digestive disorder (saponins, tannins and flavanoids), anti-oxidant (polyphenols), anti-plasmodial (treptamine, tannins, organic acids and saponins. These bioactive compounds unswervingly countered with cells of effected tissue of host and influenced them in a specific manner such as: a triterpnoid (avicin) isolated from *Acacia victoriae* has inhibited the tumor cell growth and induced apoptosis by perturbing mitochondria functions in effected host cell (Haridas et al*.,* 2001). Unlike the avicin, these bioactive compounds can also manipulate with salt channel on the cell membrane and reverse the permeability of membrane (Gilani et al., 1999). It was also observed that they act as antagonist or inhibitor of a particular enzyme or protein, for instance two terpenoids isolated from *A. catechu* L. act as antagonist of nucleotidase enzyme in *Streptococcus mutants* MT 8148 (c) and *S. mutants* MT 6715 (g) (Iwamoto et al*.,* 1991).

The phytochemical study of *A. nilotica* and *A. catechu* were also carried out in present exploration which concluded that bark and pods of *A. catechu* contained maximum plant metabolite inluding: alkaloids, tannins, saponin, flavonoids, and carbohydrates. In contrast to *A. catechu*, the *A. nilotica* contains less number of plant metabolites.

Conclusion

Present study concluded that *A. nilotica* and *A. catechu* showed their potential antimicrobial activities among the investigated species, and provide an ample opportunity to plant based drug deigning due to their considerable role in the ethnomedicine, most effectiveness against various microbial pathogens and their significant phytoconstiuents.

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