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Antimicrobial and phytochemical screening of *Trikuta*- traditional food of western Rajasthan

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Dried plant products of North west Rajasthan which are cooked as a vegetable known as *Trikuta*-seeds of *Acacia Senegal* (L.) Willd., unripe fruits of *Capparis deciduas* (Forssk.) Edgew. and unripe pods of *Prosopis cineraria* (L.) Druce were tested against seven clinical isolates including one Gram positive and six Gram negative bacteria using Agar well diffusion method. Methanolic extract of unripe pods of *Prosopis cineraria* and unripe fruits of *Capparis decidua* showed excellent antimicrobial activity against all the clinical isolates, where as seeds *of Acacia senegal* showed inhibition against five bacterial strains. The lowest MIC values were recorded by the unripe pods of *Prosopis cineraria* in comparison to unripe fruits of *Capparis decidua* and seeds of *Acacia senegal*. The Phytochemical analysis showed the presence of tannins, alkaloids, flavonoids and glycosides in the *Prosopis cineraria* and presence of alkaloids, saponins, glycosides in *Capparis deidua* whereas tannins, alkaloids, steroids, flavonoids and glycosides was evaluated in seeds of *Acacia senegal*. The study depicts that dried plant products of western Thar Desert possess medicinal properties.

Keywords: Antimicrobial, Phytochemical, *Trikuta*

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Increasing resistance in pathogenic microbes against present antibiotic and toxicity in human being is a great problem. This shift in susceptibility greatly affects our ability to successfully treat patients empirically[1]. Interest in plant and plant products has revived due to the over prescription and misuse of antibiotics and resistance to these drugs by microorganisms[2]. Plant products have been valued for ages as remedies for human diseases. They provided the material used in various systems of alternative medicine such as *Unani*, *Ayurveda*, *Siddha*. Some dried products like pepper corns (*Piper nigrum* L.), nutmeg (*Myristica fragrans* Houtt.), *ajwain* (*Trachyspermum ammi* Sprague.), turmeric (*Curcuma domestica* Valeton.), clove (*Syzygium aromaticum* L. Merrill & Perry) are well known for their antiseptic, anti-inflammatory, anti-allergy antibacterial properties from ancient time. These dried products are the richest bio-resources of drugs for the medicinal system[3]. The medicinal properties exhibit by dried plant products is due to presence of secondary metabolites in it. Diverse range of secondary metabolites such as terpenoids, alkaloids, polyacetylenes, flavonoids, steroids and glycosides was studied in dried plant products and now they are use in the modern drugs for curing various diseases. These dried plant products are also used by local people in curing toothache, anti-allergic, snakebite antidote, paralysis, etc.[4] This reveals that these dried plant products possess nutritional as well as medicinal properties and have a great potential for producing new drugs for human benefits. Therefore, in the present study we have investigated the antibacterial property and phytochemical study of some specific dried products of western Rajasthan which are, yet not properly investigated. Western part of Thar Desert where famine occurs year after year due to the failure of monsoon, man has to depend upon many indigenous plant species for his food resources. Due to the droughts, many plants and specially dried plant products are used as a famine food.

Materials and methods

Collection of dried products

Dried plant products, viz. Seeds *of Acacia senegal* (L.) Willd. (local name: *Kumbat, Kumbatiyo*), unripe fruits of *Capparis decidua* (Forsk.) Edgew. (local

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name: *Ker, Kerro*) and unripe pods of *Prosopis cineraria* (L.) Druce (local name: *Khejari*) were collected from the market of Jodhpur, Rajasthan and identified with the help of Botanical Survey of India (BSI), Jodhpur, Rajasthan and Flora of South Central Rajasthan[5]. Specimens were deposited at Mycology and Microbiology unit of Botany department, Jai Narain Vyas University, Jodhpur as Voucher number MM1001 *Acacia senegal*, MM1002 *Capparis decidua*, MM1003 *Prosopis cineraria*, respectively.

Preparation of extracts

Dried products were powdered and 25 gm of powder was used for extraction using Soxhlet apparatus. The extraction was made with different solvents on the basis their increasing polarity of, viz. petroleum ether, chloroform, and methanol. The resultant extracts were placed in rotary evaporator to evaporate the solvents and kept in vacuum desiccators for further study. Aqueous extract (water) of dried plant products was prepared by reflux method. 20 gm of powder was heated with 200 ml of distil water at 50 °C under reflux for 48 hrs[6].

Bacterial culture

The antibacterial activity was tested against seven clinical isolates. It includes one gram positive s*taphylococcus aureus* and six gram negative bacteria *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Citrobacter koserii, Morgenellam organii, Citobacter frundii*. These isolates were taken from the Ramdeo laboratory, of Jodhpur, Rajasthan and were identified microscopically and on biochemical basis[7]. Identified isolates were stored in 20 % glycerol at -20 °C and subculture on the nutrient agar at 37 °C for 24 hrs before use.

Inocula preparation

Bacterial strains were inoculated into 10 ml of sterile nutrient broth, and incubated at 37 °C for 24 hrs. The concentration of inoculum was set to 0.5 McFarland's standards[8].

Antibacterial susceptibility assay

The antimicrobial susceptibility was determined by using Agar Well Diffusion Method[9].The entire nutrient agar surface was seeded with the inoculums suspension and was allowed to dry for 5 min. The wells of 6 mm were created and 70 µl of the each extract was poured into it. The plates were kept in refrigerator for about 15 min for proper diffusion of the extract and then were incubated at 37 °C for 24 hrs. At the end of incubation zone of inhibition was measured in millimeter. This exercise was done in triplicates to ensure reliability.

Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) was determined by Agar well diffusion method[10]. Stock solutions of extracts were prepared. It was then diluted by two-fold dilution method to achieve the required concentrations i.e. 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml, 3.12 mg/ml, respectively**.** Agar plates were prepared and seeded with 100 µl of bacterial suspension. Well were made with the help of sterile cork borer and 70 µl of each dilution was added into the well. Plates were incubated at 37 °C for 24 hrs. The lowest concentration of the extract where visible growth of micro-organism was not seen is considered as MIC.

Analysis of variance (ANOVA)

The experimental data from triplicates were analysed through ANOVA by Strip plot design. The clinical isolates (n = 7) were named as vertical factor and the extracts were named as horizontal factor (n = 4).

Phytochemical screening

Preliminary phytochemical analysis of the dried plant products were undertaken and tested for various antimicrobial phytoconstitutents[11-12].

Alkaloids

About 200 gm of plant material was boiled with 2 % HCl on a steam bath and filtered. 1 ml portion of the filtrate was measured into 4 test tubes. Each of the 1 ml filtrate was treated with 2 drops of the following reagents for the presence of alkaloids.

1) Mayer's reagent (Potassium mercuric iodide solution): gives creamy-white colored precipitate.
2) Dragendorff's reagent (Potassium bismuth iodide solution): gives red precipitate.
3) Wagner'sreagent (Iodine potassium iodide solution): gives reddish-brown precipitate.
4) Hager's reagent (Picric acid and Picrolonic acid): gives yellow colored precipitate.

Tannins

About 200 mg of powdered plant material was boiled in 10 ml of distil water. The mixture was filtered**.**

To the 2 ml of the filtrate, 2 ml of $FeCl_3$ solution was added and observed for greenish to black colour.

Saponins

About 100 mg of sample was boiled with 5 ml of distil water. The mixture was filtered and 1ml of the filtrate was shaken vigorously and observed for froth formation.

Flavonoids

Two methods were used to determine the presence of flavonoids in plant sample:

1. About 200 mg of plant material was dissolved in diluted NaOH and then HCL was added to it. A yellow solutions turns to colorless, show the presence of flavonoids.
2. To the 200 mg of plant material and 10 ml ethanol was added .The mixture was boiled for few min. A small piece of magnesium ribbon was added to the filterate with addition of few drops of conc. HCl. Pink tomato red colour indicates the presence of flavonoids.

Steroids and Terpenoids

A quantity (9 ml) of ethanol was added to 1 gm of the extract and refluxed for a few minute and filtered. Each of the filtrates was concentrated to 2.5 ml in a boiling water bath. Distilled water, 5 ml was added to the concentrated solution, the mixture was allowed to stand for 1 hr and the waxy matter filtered off. The filtrate was extracted with 2.5 ml of chloroform using a separating funnel. To 0.5 ml of the chloroform extract in a test tube, 1 ml of concentrated sulphuric acid was carefully added to form a lower layer. A reddish brown interface shows the presence of steroids. To another 0.5 ml of the chloroform extract was evaporated to dryness on a water bath and heated with 3 ml of concentrated sulphuric acid for 10 min on a water bath. A grey colour indicates the presence of terpenoids.

Glycosides

Keller-kiliani test –To 2 ml of the filtrate, 1ml of Glacial acetic acid, $FeCl_3$ and conc.H_2SO_4 was added. A green blue colour indicates the presence of glycosides.

Results

Preliminary screening of antimicrobial potential was evaluated by using Agar well diffusion method on different extracts of dried plant products is presented in Table 1. Among the selected dried plant products, unripe pods of ***Prosopis cineraria*** is found more effective then fruit of ***Capparis decidua*** and seeds of ***Acacia senegal*** in inhibiting the growth of pathogens. Maximum activity was recorded by methanolic extracts followed by chloroform extracts, whereas no activity was recorded by petroleum ether extracts. Methanolic extracts of unripe pods ***Prosopis cineraria*** and fruits ***Capparis decidua*** shows significant results on all pathogens namely ***Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Psedomonas aeruginosa ,Citrobacter koserii, Morgenellam organii, Citobacter frundii*** and

Table 1—Antimicrobial activity of dried plant products

Dried plant product	Plant extracts in organic solvents	Diameter of inhibition zone in mm						
		S. aureus	*E. coli*	*K. pneumoniae*	*P. aeruginosa*	*C. koserii*	*M. morganii*	*C. frundii*
A. senegal (seeds)	Aq.	-	-	-	-	-	-	-
	Met.	14.0±0.57	29.3±1.15	28.3±0.57	-	14.3±0.57	34.0±1.0	-
	Chlo.	-	19.6±0.57	15.6±0.57	-	-	-	
	P.E	-	-	-	-	-	-	-
C. decidua (unripe fruits)	Aq.	-	-	-	-	-	-	-
	Met	19.6±0.57	11.6±0.57	21.0±1.0	17.3±0.57	39.3±1.15	29.0±1.0	14.3±0.57
	Chlo.	-	13.6±0.57	14.3+0.57	-	-	-	12.0±1.0
	P.E	-	-	-	-	-	-	-
P. cineraria (unripe pods)	Aqu.	-	-	6.3±0.57	-	-	-	-
	Met.	35.6±0.57	29.3±0.57	34.0±1.0	19.6±0.57	39.3±0.57	37.6±1.5	21.3±1.15
	Chlo.	8.3±0.57	-	-	-	39.6±0.57	-	-
	P.E	-	-	-	-	-	-	-
Antibiotic (Chloramphenicol)		14.5±0.57	10.0±0.57	12.0±1.0	15.0±1.0	16.6±0.57	16.3±0.57	17.7±0.57

Mean ± Standard deviation, - =No activity, Aq=Aqueous, Met = Methanol, Chlo = Chloroform, PE= Petroleum ether,

maximum inhibition was observed in *Citrobacter koserii* (39.3 ± 0.57 mm) whereas methanolic extract of seeds of *Acacia senegal* showed the results on five test pathogens. The maximum inhibition by seeds of *Acacia senegal* was recorded in *Morgenella mroganii* (34.0 ± 1.0) and no activity was reported against *Pseudomonas aouriginosa* and *Citrobacter frundii*. Standard antibiotic, Chloramphenicol was taken as a positive control. It was observed that all the extracts showed better results in comparison to standard antibiotic. Table 2 represents the Minimum inhibitory concentration of most active methanolic extracts of dried products on pathogens. The lowest MIC value 3.12 mg/ml of *Prosopis cineraria* and 6.25 mg/ml of *Capparis deciduas* was recorded against *Citobacter koserii* whereas 12.5 mg/ml was the lowest MIC of *Acasia senegal* recorded against *Klebsiella pneumonia.* The result of ANOVA by Strip Plot Design method showed that all the results obtained are most significant (Tables 3-5**)**. The results of ANOVA were analyzed on the basis of Hoshmand[13]. Phytochemical analysis for various antimicrobial phyto-constitutents show the presence of tannins, alkaloids, flavonoids and glycosides in the *Prosopis cineraria* of and presence of alkaloids, saponins, glycosides in *Capparis deidua* whereas tannins, alkaloids, steroids, flavonoids and glycosides is evaluated in seeds of *Acacia senegal* Table 6.

Table 2—Minimum Inhibitory Concentration of maximum active methanol extract of dried plant products against clinical isolates

Clinical Isolates	*A. senegal*(seeds) (mg/ml)	*C. decidua* (Unripe fruit) (mg/ml)	*P. cineraria* (Unripe pods) (mg/ml)
1. *S. aureus*	50.0	12.5	12.5
2. *E. coli*	25.0	25.0	12.5
3. *K. pneumoniae*	12.5	12.5	6.25
4. *P. aeruginosa*	0.0	25.0	12.5
5. *C. koserii*	50.0	6.25	3.12
6. *M. morganii*	25.0	12.5	12.5
7. *C. frundii*	0.0	25.0	12.5

Discussion

Western Thar Desert is rich in varieties of plants. *Prosopis cineraria*, *Acacia senegal* and *Capparis decidua* are boon plants of North west Rajasthan. They provide food, shelter, fodder, wood and other income generating opportunities. Dried plant products of this region are used as a food by humans and animals. They were dried and conserved for human consumption mainly as an emergency food in drought, also known as famine food of desert. In the present study, an attempt was made to find out the antimicrobial potential and phytochemical analysis of dried plant products consumed by the people of North west Rajasthan. Earlier, the antimicrobial properties

Table 3—Analysis of variance in Strip –plot design of the sample *Acacia Senegal*

Source of variation	Degree of freedom	Sum of squares	Mean of squares	Computed 'F'
Replication	2	7.128906E-02	3.564453E-02	.5615385
Bacteria	6	5613.453	935.5754	14738.91**
Error a	12	.7617188	6.347656E-02	5415.187**
Extracts	3	4190.953	1396.984	1348.426**
Error b	6	1.547852	.2579753	
Bacteria*Extract	18	4688.546	260.4747	
Error c	36	6.954102	.1931695	
Total	83	20072		

*=significant, **=most significant

Table 4—Analysis of variance in Strip –plot design of the sample *Capparis deciduas*

Source of variation	Degree of freedom	Sum of squares	Mean of squares	Computed 'F'
Replication	2	.3095703	.1547852	.9178429
Bacteria	6	446.9763	74.49606	441.7457**
Error a	12	2.023682	.1686401	5522.854**
Extract	3	7297.286	2432.429	1067.279**
Error b	6	2.642578	.4404297	
Bacteria*Extract	18	1969.88	109.4378	
Error c	36	3.691406	.1025391	
Total	83	13324		

*=significant,**=most significant

Table 5—Analysis of variance in Strip –plot design of the sample *Prosopis cineraria*

Source of variation	Degree of freedom	Sum of squares	Mean of squares	Computed 'F'
Replication	2	.5	.25	1.384407
Bacteria	6	2100.619	350.1032	1938.742**
Error a	12	2.166992	.1805827	6026.647**
Extract	3	13130.32	4376.774	836.3838**
Error b	6	4.357422	.726237	
Bactera*Extract	18	3194.431	177.4684	
Error c	36	7.638672	.2121853	
Total	83	27513		

*=significant, **=most significant

Table 6—Phytochemical analysis of dried plant products

Compounds	*A.senegal* (seeds)	*C. decidua* (unripe fruit)	*P.cineraria* (unripe pods)
Tannins	+	-	+
Alkaloids	+	+	+
Saponin	-	+	-
Steroids	+	-	-
Flavonoids	+	-	+
Terpenoids	-	-	-
Glycosides	+	+	+

(+) presence and (–) absence of Phytochemical

were reported by researchers from stem bark[14] and leaflets[15] of *Prosopis cineraria* and even from root, stem, bark, pods of the different species of *Prosopis*, i.e., *Prosopis julifera*[16] and *Prosopis africana*[17]. Similarly, antibacterial activity was reported on respiratory tract pathogenic bacteria from stem, bark and gum extract of *Acasia senegal*[18-19]. Though unripe pods of *Prosopis cineraria* and seeds *Acacia senegal* are found in profusion and contain nutritional qualities like protein, sugar, carbohydrate, fat[20] but still no literature was found on its antimicrobial properties. However, the antimicrobial, antihelminthic, aphrodisiac, carminative, hypocholesteromic effect of different plant parts of *Capparis decidua*[21-25] has been reported previously but still the literature is skimpy from its unripe dried fruit and no earlier study was made on clinical isolates like *Citrobacter koserii, Citrobacter frundii, Morgenella morganii*. In our investigation the three dried plant products were screened for their antimicrobial property against clinical isolates and it was observed that methanolic extracts of the all dried plant product showed more significant result on pathogenic bacteria in comparison to standard antibiotic. As the three dried plant products showed the presence of alkaloids and glycosides. So, antimicrobial active chemical was supposed to belongs to these categories. Although in case of *Acasia senegal* and *Prosopis cineraria* flavonoids and tannins were also present but *Prosopis cineraria* showed more activity then *Acasia senegal.* In *Capparis decidua* there was absence of flavanoids and tannin. This represents degree of antimicrobial activity is more in *Capparis decidua* then *Acasia senegal*, hence these chemicals have non or little antimicrobial potential.

Traditional significance of study to the society

Today every nation in world tries to meet the healthcare of its citizen through proper and cost effective approaches. India has a rich legacy and traditional knowledge about plants that are widely used by several Indian system of medicine[26]. Traditional knowledge of plants and plants products of Rajasthan have been reported earlier by several workers[27-29]. Generally people of Rajasthan use seeds of *Acacia senegal,* unripe fruits of *Capparis decidua* and unripe pods of *Prosopis cineraria* to make traditional vegetable known as *Trikuta*. *Acacia senegal,* which is commonly known as *kumbhat*, is the largest producer of gum Arabic. Traditionally plant is used by local people to treat bleeding, diarrhea, leprosy, typhoid fever, bronchitis[30]. Whole plant of *Capparis decidua* is having considerable nutritional value. Medicinal use of *Capparis* is also mentioned in books like *Shushrut*, *Dhanwantri*, *Nighantu*, *Kshem*, *Kutulhan* and *Madanpal*[4]. Fruits are extensively nutritive value it is used in diet as vegetable and pickle. Bark, stem, leaves, roots, green fruit of *Capparis decidua* have a potential to cure asthma, cough, ear infection, rheumatism and ulcer[31-32]. *Prosopis cineraria*, which is also known as queen of desert or *Kalpataru* is well known to possess anthelmintic, antibacterial, antifungal, antiviral, anticancer and several other pharmacological properties[33]. Unripe green pods of *Prosopis cineraria* commonly known as *sangari* are rich in protein and

fiber content. Flour of dried pods is believed to help in blood purification and skin diseases[34]. Therefore, in the present study these three dried plant products which are abundantly available and usually consumed by the people as food of Western Rajasthan were investigated for screening its antimicrobial and phytochemical properties. The study reveals that, undertaken dried plant products showed significant antimicrobial property against clinical pathogens in comparison to standard antibiotic. This showed that dried plant products have great potentiality compared to commercially available antibiotics. Consumption of these dry products may build up the immumity against pathogens. The presence of phytochemicals in the studied dried products showed that antimicrobial active chemicals were supposed to belongs to these categories. The investigated dried products can be stored for longer period of time due to the presence of phytochemicals in them. Therefore, the study suggests that not only fresh and raw plant products of *Acacia senegal*, *Capparis decidua* and *Prosopis cineraria* posses economic importance but their dried products also have great nutritional as well as medicinal properties for producing new drugs for human benefits.

Conclusion

The study concluded that unripe pods of *Prosopis cineraria*, seeds *Acacia senegal* and dried fruits of *Capparis decidua* showed antimicrobial activity but unripe pods of *Prosopis cineraria* was found more pronounced then dried fruit of *Capparis decidua and* seeds *Acacia senegal*. Further, studies are required to find out more activities of these dried plant products and to isolate and identify the compounds responsible for antibacterial activity. As this will provide an ample opportunity to plant based drugs is deigning due to their effectiveness against various pathogens and their significant phytoconstituent.

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