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In vitro antibacterial activity of sixteen medicinal plants collected from nearby region of Junagadh, Gujarat (India)

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Abstract
Evaluation of phytochemical analysis and antibacterial activity of few medicinal plants collected from nearby area of Junagadh, Gujarat, India was carried out. Presence of phytochemicals in different types of extracts (chloroform, alcoholic and aqueous) was evaluated as per standard chemical methods. All extracts were evaluated for having antibacterial action using disc diffusion assay with selected bacterial cultures. Out of 16 medicinal plants methanolic extract of *Adansonia digitata* L. leaves showed a zone of inhibition against *Klebsiella pneumoniae* (20.02±0.08 mm) and *Salmonella typhi* (20.69±0.08 mm). Methanol and water extracts of *Ficus racemosa* L. bark showed a zone of inhibition of 18.48±0.02 mm and 19.68±0.03 mm, respectively against *Escherichia coli*. Whereas chloroform and methanol extracts of *Pueraria tuberosa* (Willd.) DC tuber has showed a zone of inhibition of 20.68±0.03 mm and 19.73±0.3 mm, respectively against *Klebsiella pneumoniae* and *Streptococcus agalactiae*. Phytochemical screening of all the plants revealed the presence of various phytochemicals like alkaloid, flavonoids, steroidal compounds etc. The result from the study may be useful for further research on bio-prospecting.

Keywords: Medicinal plants, chloroform, alcoholic, phytochemical analysis and antibacterial activity

Introduction
The current antibacterial therapy is substantially threatened due to the emergence and spreading of multidrug-resistant (MDR) bacterial pathogens [1]. Infection due to MDRs often leads to increased mortality, longer time of therapy and higher cost of treatment with the cost of the health of animals [1, 2]. A number of pathogenic organisms includes but not limited to *Escherichia coli* (EC), *Klebsiella pneumoniae* (KP), *Staphylococcus aureus* (SA) etc. are going to become antibiotic-resistant due to repeated and overuse of different classes of antibiotics [2-4]. A number of scientific communities have declared the EC, KP, SA and other pathogenic bacteria as notorious pathogens which may require newer bacterial drug [1, 4]. Increase in antibiotic resistance in bacteria leads to use higher classed and expensive antibiotic drugs or previously discarded drugs which associated with significant side effects and compromising patient's health [1]. Therefore, it is very necessary to explore other alternative antibacterial therapeutic agents which can be potentially used in the treatment as well as eradication of harmful pathogenic bacteria.
The usefulness of medicinal plants with antibacterial activity as well as is documented in various ancient systems of medicines like Ayurveda, Chinese medicine and Unani medicine. World Health Organization has also suggested the use of traditional medicine and phytomedicine in various diseases conditions [5]. A large number of medicinal plants are found in the Saurashtra region of Gujarat (India). People from this region are commonly using a number of medicinal plants since a long time for the primary healthcare of their domestic animals. So, it is very important to evaluate and document the antibacterial activity or potential of plants in terms of their *in vitro* and *in vivo* efficacy [6].
In this study, we aimed to determine the *in vitro* antibacterial activity of various extracts from some selected medicinal plants from the surrounding area of Junagadh against selected pathogenic bacteria by disc diffusion assay. We selected 16 medicinal plants after the preliminary screening to determine their *in vitro* antibacterial potential (Table 1).

Materials and methods

Collection and processing of plant material

All plant materials listed in table 1 were collected from surrounding regions of Junagadh, Gujarat (India). Collected plant materials were identified and authenticated by Mr. Punit Bhatt, Pharmacognosist. Collected plant materials were washed with tap water followed by shade drying. The material was used to make fine powder and stored in an air-tight container until use.

Preparation of extracts

Fine powder of each plant material defatted using n-hexane by soxhlet apparatus to remove chlorophyll and other non-polar debris. Defatted plant material was dried in an oven at 45^0C for 1 to 2 hours. About 50 g of plant material was extracted with 500 mL of chloroform, methanol and water separately at least two times. The content was filtered off and solvents were evaporated under reduced pressure using rotary vacuum evaporator below 50°C. The extracts were collected; yield was calculated and stored at 4°C for further use.

Phytochemical screening

Qualitative phytochemical screening was performed for each extract as per standard procedures [7].

Bacterial cultures

Cultures of *Bacillus cereus* (ATCC 11778), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (NCIM 5082), *Salmonella typhimurium* (ATCC23564), *Streptococcus agalactiae* (NCIM 2401) and *Staphylococcus aureus* (ATCC9144) were procured from National Chemical Laboratory (NCL), Pune.

Antibacterial activity

The antibacterial activity of different extracts and antibacterial drugs was carried out by disc diffusion assay [8]. Water and methanol extracts were reconstituted in sterile distilled water and chloroform extract was dissolved sterile distilled water and dimethyl sulphoxide (DMSO) at a concentration of 200 mg/mL. A solution of extracts (25 µL) was dispensed on blank sterile discs (6 mm diameter) and sterilized under exposure to UV light for 30 minutes. Standard antibacterial discs (Gentamicin 10 µg, Tetracycline 30 µg, Levofloxacin 5 µg, Ceftriaxone 30 µg) were also used to observe the sensitivity of bacteria. Antibacterial activity was evaluated in terms of measuring Zone of inhibition in millimetre (mm) with digital Vernier calliper (Mitutoyo, Japan).

Statistical analysis

All the data were expressed in Mean ± S.E. (n=3). The data was set up in Microsoft Excel 2013.

Results and Discussion

Per cent yield and appearance of each plant extracts are showed in table 2. Phytochemical screening of selected plant extracts is showed in table 3. In the present investigation, almost all plants showed the presence of flavonoid and phenolic compounds (polyphenol and tannin). Apart from this, *P. tuberosa*, *S. xanthocarum*, *M. oleifera*, *C. anthelminticum* and *A. digitata* exhibited the presence of alkaloid in different extracts.

Out of 16 medicinal plants, 12 medicinal plants showed an antibacterial activity. Their zone of inhibition for each extract against different bacteria is depicted in table 4. The methanol extract of *A. digitata* leaves showed the highest zone of inhibition 20.02±0.08 mm which was found comparable to that of ceftriaxone, gentamicin, levofloxacin and tetracycline against *K. pneumoniae*. Antimicrobial activity of *A. digitata* extracts against the tested bacteria could be due to the presence of alkaloid as it has previously been reported to possess antimicrobial activities [9].

A. longa is a widely distributed medicinal plant. The plant bears various phytochemicals like flavonoids, alkaloids, saponin and tannins but aristolochic acid (a phenanthrene acid derivative) is a principle cytotoxic phytochemical which is responsible for the antibacterial action of the plant [10]. In the present investigation, methanol extract *A. longa* leaves showed a zone of inhibition of 18.35±0.11 mm against *K. pneumoniae* while water extract showed the lowest zone of inhibition 11.50±0.16 mm. The methanol extract of *A. longa* leaves was found to be comparable to that of standard antibacterial drugs against *K. pneumoniae*. Extracts of the plant did not show any activity against *B. cereus and S. agalactiae.*

The water extract of *B. variegata* bark exhibited a zone of inhibition of 13.49±0.30 mm, 13.32±0.12 mm and 12.37±0.22 mm against *B. cereus*, *E.* coli and K. *pneumoniae*, respectively, while did not produce zone of inhibition with *S. typhi*, *S. aureus*, *S. agalactiae*. The plant contains major chemical constituents such as tannins, saponin, flavones, a flavonol glycoside, triterpene, phenanthraquinone. Due to the presence of these phytochemicals, plant might exhibit antibacterial activity [11].

The chloroform extract of *C. anthelminticum* seed exhibited good *in-vitro* activity and recorded zones of inhibition were 19.56±0.28 mm against *K. pneumoniae* and 11.34±0.09 mm against *E. coli*, while did not show zone of inhibition against *B. cereus, S. typhi, S. aureus and S. agalactiae.* The seeds bear avenasterol, a phytosterol which is an active principle of the seed [12]. Seeds also contain triterpenoid saponins [13] which might be responsible for its antibacterial action.

The methanol and water extract of *F. racemosa* bark showed a zone of inhibition of 18.48±0.02 mm and 19.68±0.03 mm, respectively against *E.coli*. A number of phytochemicals have been isolated from the bark of *F. racemosa* but berganin is a principal constituent [14]. Berganin showed antibacterial potential against pathogenic bacteria [15]. *M. oleifera* is a traditional medicinal plant. Leaves are used in traditional medicine as well as nutrient. Variety of phytochemicals like benzyl glucosinolates and 4-(4-O-acetyl--L-rhamnopyranosyl oxy) benzyl thiocyanate and 4-(-L-rhamnopyranosyl oxy) benzyl isothiocyanate which are known to possess antibacterial activity. Apart from this, alkaloids like moringinine and moringine also have an antibacterial action [16]. In the present investigation, chloroform and methanol extracts of *M. oleifera* leaves showed zones of inhibitions of 15.90±0.03 mm and 13.93±0.02 mm against *E.coli*, respectively. The methanol extract exhibited a zone of inhibition of 12.24±0.03 mm, which was comparable to the zone of inhibition of ceftriaxone and tetracycline against *K. pneumoniae.*

Psoralea corylifolia is traditionally used in the skin disorder. In the past decades, the extracts of the plant have been demonstrated various pharmacological actions like antibacterial, antifungal, antioxidant etc. Various coumarin derivatives and flavonoids have been identified from the plant [17]. In the present investigation, the methanol extract of *P.*

corylifolia seed showed a zone of inhibition of 11.17±0.02 mm which is similar to the zone of inhibition of ceftriaxone against *E. coli*. The chloroform and methanol extracts of *P. corylifolia* seed exhibited a zone of inhibition of 13.64±0.03 mm and 14.40±0.05mm, respectively against *S. aureus*.

The chloroform extract of *P. pterocarpum* bark showed a zone of inhibition of 14.47±0.08 mm which was higher than the zone of inhibition of ceftriaxone and tetracycline against *K. pneumoniae.* The antibacterial activity of bark might be due to the presence of diverse phytochemicals like sterol derivatives and flavonoids. This finding supports the traditional use of *P. pterocarpum* bark in dysentery and eye infection [18].

P. tuberosa as an economical medicinal plant and its antimicrobial properties of methanol and ethanol extracts of *P. tuberosa* tuber against various bacteria [19]. In the present study, the chloroform extract of *P. tuberosa* tuber showed a zone of inhibition of 20.68±0.03 mm against *K. pneumoniae.* The chloroform, methanol and water extracts showed a zone of inhibition of 17.27±0.02 mm, 14.37±0.03 mm and 11.87±0.02 mm, respectively, which were comparable to zone of inhibition of gentamicin against *S. aureus.*

The methanol and water extract of *S. xanthocarpum* showed a zone of inhibition of 15.22±0.07 mm and 17.62±0.06 mm, respectively against *E.coli*. The chloroform extract has also shown a zone of inhibition of 16.47±0.03 mm which was almost the same as a zone of inhibition of levofloxacin against *K. pneumoniae.* A possible reason for this antibacterial activity of *S. xanthocarpum* might be the presence of glycol-alkaloid like solasodine, phenolics and flavonoids in its leaves [20].

S. cumini leaves are rich in tannin and other polyphenol compounds like gallic acid and ellagic acid which have antibacterial action. Some acylated flavonol glycosides, kaempferol etc. are also reported from the plant [21]. The water extract of *S. cuminii* leaves showed a zone of inhibition of 13.31±0.10 mm against *E.coli.* The chloroform extract also showed a zone of inhibition of 14.84±0.07 mm against *E.coli.*

Presence of iridoid glucoside, tecomelloside, β-sitosterol etc might be responsible for the antibacterial action of *T. undulata* [22]. In the present investigation, the methanol extract of *T. undulata* bark showed a zone of inhibition of 15.03±0.05 mm which was comparable to the zone of inhibition of ceftriaxone against *E. coli.* The methanol extract of *T. undulata* bark showed a zone of inhibition of 9.0±0.02 mm and 9.42±0.04 mm, *S.typhi* (7.56±0.22 mm).

Table 1: List of selected medicinal plants for evaluation of antibacterial activity

S. No.	Plant species	Family	Local name (Gujarati)	Part of plant used
1	*Adansonia digitata* L.	Bombacaceae	Gorakh ambali	Leaves
2	*Aristolochia longa* L.	Aristolochiaceae	Kidamari	Leaves
3	*Bauhinia variegata* L.	Caesalpiniaceae	Kachnar	Stem bark
4	*Bauhinia variegata* L.	Caesalpiniaceae	Kachnar	Leaves
5	*Bryophyllum pinnatum* (Lam.) Oken	Crassulaceae	Paanfuti	Leaves
6	*Cassia tora* L.	Calsalpiniaceae	Kuvadiyo	Leaves, seed
7	*Centratherum anthelminticum* (L.) Kuntze	Asteraceae	Kali-jiri	Seed
8	*Euphorbia nivulia* Buch.-Ham.	Euphorbiaceae	Dandaliyo thor	Stem
9	*Ficus racemosa* L.	Moraceae	Umaro	Stem bark
10	*Moringa oleifera* Lam.	Moringaceae	Saragavo	Leaves
11	*Psoralea corylifolia* L.	Fabaceae	Bavchi	Seed
12	*Peltophorum pterocarpum* (DC.) K.Heyne	Caesalpiniaceae	Pilo-gulmohar	Leaves, Stem bark
13	*Pueraria tuberosa* (Willd.) DC.	Fabaceae	Fagiyo	Tuber
14	*Solanum xanthocarpum* Schrad. & H. Wendl.	Solanaceae	Bho-ringani	Aerial Part
15	*Syzygium cuminii* (L.) Skeels	Myrtaceae	Kala-jambu	Leaves
16	*Tecomella undulata* (Sm.) Seem.	Bignoniaceae	Ragat rohido	Stem bark

Table 2: Percent yield and appearance of the different extracts of various medicinal plants

Name of plant	Extract	Appearance	% Yield
Adansonia digitata Leaf	CE	Green sticky mass	2.04
	ME	Green mass	8.04
	WE	Dark brown solid	6.16
Aristolochia longa Leaf	CE	Green sticky mass	2.32
	ME	Brownish mass	8.48
	WE	Dark brown solid	12.36
Bauhinia variegata Leaf	CE	Light green sticky mass	3.68
	ME	Brownish mass	6.12
	WE	Dark brown solid	9.32
Bauhinia variegata Bark	CE	Green sticky mass	1.80
	ME	Dark red mass	4.40
	WE	Red solid mass	13.00
Bryophyllum pinnatum Leaf	CE	Light green sticky mass	0.88
	ME	Brownish mass	6.40
	WE	Dark brown solid	8.48
Cassia tora Leaf	CE	Green sticky mass	3.48
	ME	Brownish mass	6.60
	WE	Dark brown solid	8.76
Cassia tora seed	CE	Yellowish sticky mass	2.08
	ME	Brownish mass	4.24
	WE	Dark brown solid	9.16

Centratherum anthelminticum seed	CE	Light green sticky mass	2.64
	ME	Brownish mass	7.56
	WE	Dark brown solid	11.36
Euphorbia nivulia Stem	CE	Yellowish sticky mass	2.32
	ME	Brownish mass	8.52
	WE	Dark brown solid	10.52
Ficus racemosa Bark	CE	Colorless sticky mass	2.24
	ME	Dark red mass	6.48
	WE	Dark red slid	13.32
Moringa oleifera leaf	CE	Dark green oily	2.76
	ME	Dark green solid	5.06
	WE	Light brown mass	8.23
Psoralea corylifolia seed	CE	Dark brown oil	2.21
	ME	Dark brown solid	4.54
	WE	Brown soft mass	7.03
Peltophorum pterocarpum Leaf	CE	Green sticky mass	1.56
	ME	Brownish mass	4.96
	WE	Dark brown	9.16
Peltophorum pterocarpum Bark	CE	Colorless sticky mass	1.00
	ME	Dark red mass	6.56
	WE	Dark red slid	15.16
Pueraria tuberose tuber	CE	Yellowish sticky mass	1.96
	ME	Brownish mass	6.08
	WE	Dark brown	7.84
Solanum xanthocarpum aerial part	CE	Green sticky mass	2.04
	ME	Brownish mass	6.16
	WE	Dark brown	8.40
Syzygium cuminii Leaf	CE	Light green sticky mass	2.12
	ME	Brownish mass	8.40
	WE	Dark brown	9.60
Tecomella undulata Bark	CE	Colorless sticky mass	4.00
	ME	Dark red mass	7.20
	WE	Dark red solid	8.40

CE: Chloroform extract; ME: Methanol extract; WE: Water extract

Table 3: Qualitative phytochemical screening of different extracts of various medicinal plants

Name of plant	Extracts	Alkaloid	Glycoside	Saponin	Flavonoid	Steroid	CHO	Tannin
Adansonia digitata Leaf	CE	-	+	-	+	+	-	-
	ME	+	-	-	-	-	-	-
	WE	-	+	+	+	-	+	-
Aristolochia longa aerial part	CE	-	+	-	+	-	-	+
	ME	-	+	-	+	-	+	-
	WE	-	-	+	+	-	-	+
Bauhinia variegata bark	CE	-	-	-	+	+	-	+
	ME	-	+	-	+	+	+	+
	WE	-	+	+	-	-	+	-
Bauhinia variegata leaf	CE	-	-	+	+	-	-	-
	ME	-	+	+	+	+	+	-
	WE	-	+	+	-	-	-	+
Bryophyllum pinnatum	CE	-	-	-	-	+	-	+
	ME	-	+	-	+	-	+	-
	WE	-	+	+	-	-	+	-
Cassia tora leaf	CE	-	-	-	+	+	-	-
	ME	-	+	-	+	-	+	-
	WE	+	+	+	+	-	+	+
Cassia tora seed	CE	-	-	-	+	+	-	-
	ME	-	-	+	+	-	-	-
	WE	-	+	+	-	-	+	+
Centratherum anthelminticum leaf	CE	-	-	-	-	-	-	-
	ME	-	+	-	-	+	-	+
	WE	+	+	+	+	-	+	-
Euphorbia nivulia stem	CE	-	-	-	-	+	-	-
	ME	-	-	+	+	-	-	-
	WE	-	+	+	+	-	-	-
Ficus racemosa bark	CE	-	-	-	-	+	-	+
	ME	-	+	+	-	+	-	+
	WE	-	+	+	+	-	-	+
Moringa oleifera leaf	CE	+	-	-	+	+	-	-

	ME	+	+	-	+	+	+	+
	WE	+	+	+	-	-	+	+
Psoralea corylifolia seed	CE	-	-	-	-	-	-	-
	ME	-	+	-	+	-	-	+
	WE		+	+	+	-	+	+
Peltophorum pterocarpum leaf	CE	-	-	-	-	+	-	-
	ME	-	+	-	+	-	-	-
	WE	-	+	+	+	-	+	+
Peltophorum pterocarpum Bark	CE	-	-	-	+	+	-	-
	ME	-	+	-	+	-	+	+
	WE	-	+	+	+	-	+	+
Pueraria tuberosa tuber	CE	-	-	+	+	-	-	-
	ME	-	+	+	+	-	+	-
	WE	+	+	+	-	-	+	+
S. xanthocarpum aerial part	CE	+	-	-	+	+	-	-
	ME	+	+	-	-	+	+	-
	WE	+	+	+	+	-	-	+
Syzygium cuminii leaf	CE	-	-	-	+	+	+	-
	ME	-	-	+	+	-	+	-
	WE	-	+	+	+	-	-	+
Tecomella undulata bark	CE	-	-	-	+	+	+	-
	ME	-	+	+	+	+	-	-
	WE	-	+	+	+	-	+	+

CE- Chloroform extract; ME-Methanol extract; WE-Water extract; CHO-sugar; '+' indicates positive test; '-' indicates negative test.

Table 4: Mean zone of inhibition (mm) of different extracts of various medicinal plants against pathogenic bacteria

Name of plant	Extract/ antibiotic	*B. cereus*	*E. coli*	*K. pneumoniae*	*S. typhi*	*S. aureus*	*S. agalactiae*
Standard drugs	Gen	24.89±0.05	21.28±0.17	15.22±0.06	24.55±0.25	11.57±0.16	23.06±0.03
	Tet	24.81±0.05	27.56±0.14	11.21±0.04	33.63±0.25	29.25±0.08	31.58±0.18
	Lev	23.70±0.10	31.49±0.05	16.61±0.11	34.16±0.03	35.82±0.07	32.17±0.06
	Ctr	15.45±0.18	11.45±0.17	7.56±0.22	27.37±0.12	19.35±0.09	20.91±0.03
A. digitata leaf	CE	nd	nd	nd	8.1±0.02	nd	nd
	ME	nd	nd	20.02±0.08	20.69±0.08	10.21±0.06	nd
	WE	nd	nd	nd	nd	nd	nd
A. longa leaf	CE	nd	nd	12.85±0.26	15.81±0.09	nd	nd
	ME	nd	13.61±0.26	18.35±0.11	15.37±0.28	14.21±0.14	nd
	WE	nd	nd	11.50±0.16	nd	nd	nd
B. variegata bark	WE	13.49±0.30	13.32±0.12	12.37±0.22	nd	nd	nd
C. anthelminticum seed	CE	nd	11.34±0.09	19.56±0.28	nd	nd	nd
F. racemosa Bark	CE	nd	nd	7.47±0.02	7.62±0.05	nd	nd
	ME	nd	18.48±0.02	nd	nd	nd	nd
	WE	nd	19.68±0.03	7.62±0.03	7.17±0.02	nd	nd
M. oleifera leaf	CE	nd	15.90±0.03	nd	16.62±0.05	nd	13.89±0.04
	ME	nd	13.93±0.02	12.24±0.03	nd	nd	nd
	WE	nd	Nd	nd	14.34±0.04	nd	nd
P. corylifolia seed	CE	12.13±0.02	9.81±0.01	nd	9.77±0.03	13.64±0.03	nd
	ME	10.94±0.01	11.17±0.02	nd	13.54±0.02	14.40±0.05	nd
	WE	nd	nd	nd	nd	nd	nd
P. pterocarpum bark	CE	13.42±0.07	nd	14.47±0.08	nd	nd	nd
	ME	nd	nd	nd	nd	nd	nd
	WE	nd	nd	nd	13.17±0.03	nd	nd
P. tuberosa tuber	CE	nd	nd	20.68±0.03	nd	17.27±0.02	nd
	ME	nd	nd	nd	nd	14.37±0.03	19.73±0.03
	WE	nd	nd	12.13±0.04	nd	11.87±0.02	nd
S. xanthocarpum aerial part	CE	nd	nd	16.47±0.03	nd	nd	nd
	ME	nd	15.22±0.07	nd	nd	nd	nd
	WE	nd	17.62±0.06	nd	nd	nd	nd
S. cuminii leaf	CE	nd	nd	14.84±0.07	nd	nd	nd
	ME	nd	8.42±0.05	nd	14.11±0.06	nd	Nd
	WE	9.6±0.03	13.31±0.10	nd	16.29±0.08	8.13±0.02	nd
T. undulata bark	CE	nd	nd	nd	nd	nd	nd
	ME	10.23±0.04	14.52±0.02	9.0±0.02	15.03±0.05	nd	nd
	WE	nd	nd	9.42±0.04	nd	nd	nd

All the data are expressed as mean ± SE (n=3); nd= Not Detected; CE- Chloroform extract; ME-Methanol extract; WE-Water extract, Gen= Gentamicin; Tet=Tetracycline; Lev=Levofloxacin; Ctr= Ceftriaxone

Conclusion

Different extracts of medicinal plants used in the present study showed *in-vitro* antibacterial activity which might be due to presence of various phytochemicals. Further isolation and identification of phytochemicals from plants which showed remarkable antibacterial activity may be useful and can be further explored to in terms of *in-vivo* evaluation.

Conflict of interest

Authors declare no conflict of interest.

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