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Research Article

POTENTIAL ANTIMICROBIAL ACTIVITY OF THREE TOP BRANDED MARKET SAMPLES IN COMPARISON TO ORIGINAL MOTHER PLANT OF *TRIGONELLA CORNICULATA*-KASURI METHI

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ABSTRACT

Trigonella corniculata commonly known as Kasuri methi owing to its origin from a small city Kasur located to South of Lahore in the Pakistani province of Punjab has been known since ages and is very ancient spice used as flavoring agent and nutritional supplement. The small herb belongs to *Fabaceae* family and is also known as Goat "s horn and Bird "s foot. The word fenugreek in Latin means "Greek hay" as it was used mainly as fodder for animals. The leaves and seeds are considered to be the most used part and mostly used as flavoring agent in preparation of vegetables and pickles. The plant has been reported to be a rich source of dietary fiber along with proteins. The leaves are rich source of essential vitamins and minerals and have potential bacteriocidal activity for shielding the body against a range of chronic disorders. The present investigation is carried out to compare the potency of antimicrobial activity at different concentrations of original mother plant of kasuri methi to the available branded market samples of Kasuri methi. Although the adulteration of market samples to original leaf powder using molecular markers is in progress to correlate the two studies, the present investigation was carried out to analyse the phytochemicals present in methanolic extracts of mother plant and bacteriocidal potential of market and original samples.

INTRODUCTION

T. corniculata belongs to *Fabaceae* family and genus *Trigonella* which further consisted of 50 species most of which have Iranian and Indian origin. Out of eleven different species cultivated in India, *T. foenum graceum* (L.) fenugreek and *T. corniculata* are commercially available (Singhania DL *et al*., 2016)[1] and are the oldest cultivated spice crop of India. The native area extends from Mediterranean basin to South Asia while Turkey owes the pleasure of having 49 species (Davis PH *et al*. 1978)[2]. In India, Punjab and Kashmir are major area where the herb grows as wild species. Wild *T. gladiala* is considered as wild ancestor of cultivated fenugreek which may be evolved in upcoming times to new extinct forms of *T. foenum graceum*. Fenugreek is found growing in varied climates ranging from cool temperature steppe to wet tropical and dry forests of Asia, Africa, Europe, Australia and America. The herb is multipurpose and commercially important spice crop grown for its seeds, tender shoots and fresh leaves. The seeds and leaves have distinct aroma and slightly bitter taste. It is an annual plant and important food crop in India, North Africa, and Yemen. More than 68% of world production is accomplished by India being a leading producer. India is major exporter of this food crop to countries like Sri Lanka, UAE, Japan, South Africa, Nepal and Saudi Arabia (Malhotra and Rana, 2008)[3]. According to Natural database-2010, 523 commercial products and 50 Canadian licensed products contain methi and inspite of this the crop is a neglected plant and has been underuse (Sati SC *et al*. 2010)[4]. The crop is locally cultivated by people, which resulted in limited large scale cultivation. Moreover the genetic diversity information is another aspect that limits its production. The dried seeds and leaves are used as insect repellent in grain storage; the seed oil is used in perfumery in France, Leaves as greeny vegetables and seeds as spice powder and also as a fodder crop in India, Australia, Canada (Rajagopalan MS, 2001; Moyer JR *et al*. 2003; Thomas and Basu, 2008)[5,6,7]. The plant is in demand owing to a natural source of diosgenin and galactomannan in

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pharmaceutical and steroid industries. Kasuri methi leaves are rich in folic acid, thiamine, Vitamins A, B_6, C, riboflavin and niacin, whereas in mineral potassium, iron, phosphorus and calcium are major ingredients (Singh and Garg, 2006)[8]. The plant owes its importance in lowering the cholesterol levels by pulling up their HDL levels in people affected from lipid fluctuations and liver disorders. The plant has been reported in fighting against dyspepsia and curbing gastric problems along with intestinal disorders. It reduces blood sugar level and preventive in healing type-II diabetes (Karim A *et al*. 2011)[9]. It reduces the chances of unanticipitated blood clotting in heart and potent antioxidant in nature. Leaves are acclaimed worldwide to cure arthritis and atherosclerosis. Although the major constituents are same in both species except that kasuri methi is richer in iron content (Tailor and Jain, 2006)[10]. Keeping these aspects the present investigation was proceeded to compare the antimicrobial potential of original kasuri methi leaves extract to top branded market samples available easily by the name of kasuri methi leaf powder in local market.

MATERIALS AND METHODS

Plant and culture collection

The seeds of *T. corniculata* along with three top branded market samples of Kasuri methi were used in the present study for antimicrobial and phytochemical analysis. The top branded three samples of kasuri methi powder were procured from local market, Kurukshetra, Haryana, India. The seeds of *T.corniculata* were cultured following all agronomic practices. The leaves of plant were taken for investigating antibacterial activity. The human pathogenic microorganisms were procured from Microbial Type Culture Collection (MTCC) Institute of Microbial Technology (IMTECH), Chandigarh; which included Gram-negative bacteria *E. coli* (MTCC-443), *P. aeruginosa* (MTCC-424) and Gram-positive bacteria *S. aureus* (MTCC- 96) and *B. subtilis* ((MTCC- 441) and *S. mutans* (MTCC- 1943).

Preparation of T.corniculata leaves extract

Leaves of plant cultured in nursery were collected and shade dried and finally grounded to fine powder. The 10g powder was soaked in 100 ml of different solvents for 72 hours and filtered. The filtrate was evaporated at 45-50°C in water bath. The residual powder after solvent extraction was dissolved in DMSO and stored at 4°C. Similar procedure was followed for market samples brought as leaf powder.

Antimicrobial activity of different solvent extracts

The antimicrobial activities of plant leaves were evaluated by agar well diffusion assay (Perez C *et al.*1990)[11]. DMSO was used as a negative control whereas ciprofloxacin was used as positive control. The anti microbial activity of extract was determined by inhibition zone diameters. The zones were measured by high media zone scale. Comparison in activities of leaves at different concentrations was subjected to antimicrobial assay.

Phytochemical screening

The crude extract of plant leaves were subjected to qualitative phytochemical screening for identification of active chemical constituents using standard methods as described by Trease and Evans (2002)[12].

RESULTS AND DISCUSSION

Comparative analysis of antimicrobial activity

Although a number of researcher have elucidated the antimicrobial potential of aqueous and organic solvent extracts using mainly seeds and leaves as plant part in *T. foenum graceum* against number of pathogens. The present investigation is rare in comparing the potential of top branded sample of kasuri methi available in market to the original mother plant of both different species of *Trigonella* such as *Trigonella corniculata* and *Trigonella foenum graceum.* Different solvent system were used as extractant to validate the solubility of powdered samples in one particular solvent and were tested against four microbial pathogens. The agar disc diffusion method was used to evaluate the antimicrobial activity by measuring the inhibition zones against the tested pathogens. DMSO served as negative control in which no inhibition zone was observed.

The methanol has been the most exploited solvent system or say extractant to extract maximum number of phytoconstitutes which in turn were responsible for effective zones of inhibition for antimicrobial activity. Chloroform is solvent system second to methanol widely used for extraction procedures in antimicrobial assay. But first renowned market sample (MS-1) purchased as leaf powder was unable to produce any zone of inhibition in these two solvent systems (Table-1).

Table 1 Inhibition zones diameters (in mm) of samples-1(MS-1) in different solvent extracts against different pathogens (10%).

Solvents	(Conc.) mg/ml	*E.coli.*	*B.subtilis*	*S.aureus*	*P.aeruginosa*
Petroleum ether extracts	0.25	22	-	12	18
	0.5	28	-	14	14
	1.0	30	-	17	16
Hexane	0.25	15	-	-	10
	0.5	18	-	-	-
	1.0	20	12	-	12
Methanol	0.25	-	-	-	-
	0.5	-	-	-	-
	1.0	-	-	-	-
Ranksolv	0.25	-	-	-	-
	0.5	-	-	-	-
	1.0	-	-	-	-
Chloroform	0.25	-	-	-	-
	0.5	-	-	-	-
	1.0	-	-	-	-

We have tried a rarely used solvent called ransklov which again was unable to produce any effective activity against the selected microbial pathogens used in present study Sampathkumar V *et. al,* in 2008[13] also reported no zone of inhibition using different solvent extracts and further described that it may be due to loss of active component during extraction process or may be the lack of solubility of active constituent in the opted solvent. However the powdered market sample (MS-1) resulted in significant zones of inhibition using petroleum ether and hexane as solvent system at three different concentrations of 0.25, 0.5 and 1.0mg/ml which were more pronounced against gram negative bacteria *E.coli.* At a

concentration of 0.25mg/ml a zone of 22mm was observed which goes on increasing in a linear order with the increasing concentration. A zone of 28mm was observed at 0.5mg/ml which on doubling the concentration to 1.0mg/ml resulted in a zone of 30mm. While on using hexane as solvent system a zone of 15mm (0.25mg/ml) was observed which increased to 18mm at 0.5mg/ml and further increased to 20mm at 1.0mg/ml. The zone size goes on increasing linearly with increased concentration. Testing against *B.subtilis* only hexane extracts resulted in a zone of 12mm only at a higher concentration of 1.0mg/ml. Petroleum ether extracts resulted in a linear relation with zones of inhibitions to increasing concentrations against *S.aureus*. A zone of 12mm (0.25mg/ml), 14mm (0.5mg/ml) and 17mm (1.0mg/ml) were observed. The linear relationship observed so far was missing in antimicrobial activity of solvent extracts against *P.aeruginosa*. A zone of 18mm was reported at 0.25mg/ml which on increasing the concentration to 0.5mg/ml resulted in a zone of 14mm while on further increase in concentration to 1.0mg/ml produced a zone of 16mm. Hexane extracts of same sample (MS-1) resulted in zones of 10mm at 0.25mg/ml and only 12mm at 1.0mg/ml while no zone was observed at a concentration of 0.5mg/ml. Similar to our study Kroum NEH in 2009[14] carried out antibacterial activity using methanol, chloroform and aqueous extracts against standard bacterial strains and revealed that methanol and aqueous extracts are poorest solvent system in concern to fenugreek samples similar to (Al Abdeen SZ *et. al*, 2010)[15]**.** Divyesh C and his coworkers in 2013[16] also supported that petroleum ether is much better solvent system to study antibacterial activity against opportunistic pathogens in fenugreek. The present anomalies may be due to very low concentrations used in present study as very high concentrations of 125, 250 and 350mg/ml have been used by previous researchers (Elnoor MEM *et. al*, 2015)[17] where higher concentration of 250mg/ml was used in fenugreek to study antimicrobial activity.

Market purchased sample number-2 (MS-2) was tested using different solvent extracts like petroleum ether and chloroform of leaf powder were ineffective against the bacterial pathogens tested except methanolic and hexane extracts which resulted in a zone of 12mm in both (Table-2) while moderate zones of inhibitions were produced against *S.aureus* using different solvent systems.

Table 2 Inhibition zones diameters (in mm) of samples-2(MS-2) different solvent extracts against different pathogens (10%)

Solvents	*B.subtilis*	*S.aureus*	*P.aeruginosa*	*E.coli*
Methanol	12	12	-	17
Chloroform	-	14	-	**16**
Petroleum ether	-	10	08	08
Hexane	12	-	-	12
DMSO	-	-	-	-

Methanolic extract resulted in a zone of 12mm, chloroform extracts resulted in 14mm zone of inhibition while petroleum ether extracts resulted in a zone of 10mm only. (Sharma V *et. al,* 2017)[18] supported the utilization of methanol as solvent system. Their result well corroborate with our as methanolic extracts resulted in highest zone of 20mm against *E.coli*, 19mm against *S.aureus* using fenugreek leaves as plant part. Similarly acetone extracts resulted in a zone of 16mm for both *E.coli* and *S.aureus,* while aqueous extracts resulted in a zone of only 4mm against *E.coli* and 2mm against *S.aureus*. Similar to Dash BK and his coworkers[19] reported that methanol is effective in extracting those constituents which resulted in better antimicrobial activity against *Pseudomonas* sp. whereas acetone extracts were more potent against *E.coli*. Our result further correlate to Kumar G and his coassociates in (2010)[20] and Massih AR *et. al*, (2010)[21] where both gram positive and gram negative bacteria get inhibited by using methanolic extracts when compared to acetone extracts. Similar results were reported by (Premnath R *et. al,* 2011)[22] using stem, leaves and seeds where ethanolic extracts were more efficient in inhibiting pathogens using methanol as solvent system as compared to acetone. The sample resulted in low efficacy against *P.aeruginosa* where petroleum ether extracts resulted in a zone of 8mm only while all other solvent systems were unable to show any activity against different pathogens tested. The market sample was quite effective against *E.coli* a gram negative bacteria. Almost all solvent extracts resulted in different zone of inhibitions ranged from (8-17mm). Highest zone of inhibition 17mm was observed in methanolic extracts which is followed by chloroform extract (16mm) which in turn followed by a zone of 12mm using hexane as solvent system while lowest zone of inhibition of 8mm was observed using petroleum ether as solvent.

Market sample number -3 was extracted using methanol resulted in a zone of 17mm which is followed by a zone of 16mm using hexane as solvent while a zone of 14mm was observed using chloroform as a solvent system. However petroleum ether which resulted in effective zones in market sample no-1 and 2 was unable to inhibit gram positive *B.subtilis*. Only a single zone of 16mm was observed in methanolic extracts against *S.aureus* while all other solvent systems were inefficient to produce any zone of inhibition in other solvent systems. Against *P.aeruginosa* only hexane and petroleum ether extracts resulted in a similar zone of inhibition o 12mm while all other solvent did not produce any zone of inhibition (Table-3).

Table 3 Inhibition zones diameters (in mm) of samples-3(MS-3) different solvent extracts against different pathogens (10%)

Solvents	*B.subtilis*	*S.aureus*	*P.aeruginosa*	*E.coli*
Methanol	17	16	-	18
Chloroform	14	-	-	16
Petroleum ether	-	-	12	14
Hexane	16	14	12	17
DMSO	-	-	-	-

Significant results were reported in all solvent systems against *E.coli* producing zone of inhibition that ranged from 14mm to 18mm. Methanolic extracts resulted in a zone of 18mm followed by 17mm using hexane, 16mm using chloroform and a minimum of 14mm using petroleum ether as extractant. Tailor and Jain, 2006[10] revealed that leaf extracts of kasuri methi have pronounced effects against *B.subtilis* which is contradictory to present investigation .All the market samples were effective against *E.coli* a gram negative bacteria instead of gram positive *B.subtlis*. To validate the results all the three market samples and two different species of original pure plant leaves were tested against *B.subtilis* (Table-4). *B.subtilis* has

been reported to be the most sensitive bacterium which gets inhibited using different solvent extracts of methi. The experiment clearly described the efficacy of kasuri methi over green methi and market samples when compared to the different market samples along with cultured two different species of *T.corniculata* and *T. foenum graceum*.

Table 4 Inhibition zones diameters (in mm) of different samples methanolic extracts against *B.subtilis.*

Samples	10mg/ml	5mg/ml	2.5mg/ml	1.25mg/ml
Sample(MS-1)	19	14	14	14
Sample(MS-2)	-	11	15	16
Sample(MS-3)	16	14	14	12
T.corniculata (Kasuri methi)	15	15	14	11
T.foenum graceum (Simple methi)	-	-	-	-
(-) control (DMSO)	-	-	-	-

Methanolic extracts of all samples were prepared being most proven solvent system to extract maximum phytoconstitutes from leaf parts. Four different concentrations of 1.25mg/ml,2.5mg/ml,5mg/ml and 10mg/ml were prepared to test the efficacy of different samples against *B.subtilis*. Green methi (*T. foenum graceum*) was unable to produce any zone of inhibition at any of the four concentrations which might be due to lower concentration as earlier reports have been showing results at 125 and 250mg/ml concentrations. Our results are well in accordance to (Al Abdeen SZ *et al.* 2010)[15].The study analyzed the antibacterial activity of aqueous and organic extracts of different plant parts like stem, leaves, seeds and roots of fenugreek using colony count method and agar well diffusion method, but did not observed any zone of inhibition against any of the pathogen viz- *S.aureus, E.coli*, *K.spp. and P.aeruginosa.*

In comparison to green methi, kasuri methi resulted in different zones of inhibition at all four concentration tried. A highest of 15mm was observed at 10mg/ml which remain constant at 5mg/ml, 14mm at 2.5mg/ml and further resulted in a zone of 11mm at 1.25mg/ml. While on comparing the market samples highest zone of 19mm was observed at 10mg/ml which decreased to 14mm and remain constant on three lower concentrations of 5.0mg/ml, 2.5mg/ml and 1.25mg/ml. Pasricha and Gupta in 2014[23] reviewed the neutraceutical potential of methi (*T. foenum graecum*) and Kasuri methi (*T.corniculata*) using dried samples of leaves, stems and investigated the neutraceutical as well antimicrobial activity against five pathogens. Methanolic and aqueous extracts were prepared using leaves which resulted in different zones of inhibition against selected pathogens. Against *E.coli* a similar zone of 12mm was observed in both methi and kasuri methi, against *B.subtilis* a zone of 16mm using methanolic extracts and 12 mm using kasuri methi was observed however the kasuri methi aqueous extract resulted in a zone of 11mm. while against *P.mirabilis* a similar zone of 16mm was resulted using leaf extract of both meth and kasuri methi methanolic extracts. While no zone was observed against *S.aureus* and *S.epidermidis*. Market sample -2 (MS-2) contradictions was observed in relation to increased zone sizes with decreasing concentration. No zone was reported at 10mg/ml but a zone of 11mm, 15mm and 16mm were reported at 5mg/ml,2.5mg/ml and 1.25mg/ml respectively. Sample (MS-3) showed linear relationship with increasing concentration a zone of 12mm (1.25mg/ml), 14mm (2.5mg/ml, 5.0mg/ml) and 16mm at 1.0mg/ml were reported. Elnour MEM *et. al*, (2015)[17] analyzed antibacterial and phytochemical analysis using seed extracts and callus derived from hypocotyls and cotyledon explants of fenugreek using methanol and petroleum ether as solvent. Standard pathogens were opted to study antimicrobial activity against *B.subtilis, S.aureus, E.coli, P.aeruginosa, A. niger* and *C. albicans* using disc diffusion assay. Similar to our research petroleum ether comes out to be exceptional solvent system in regard to fenugreek leaf extracts. Petroleum ether extracts revealed highest zone of inhibition of 20mm against *A. niger* and 17mm against *C. albicans* in comparison to methanolic extracts which ranged from 10-12mm against *E.coli* and *S.aureus.* However the concentration used was much higher 250mg/ml as compared to present investigation.

Phytochemical Screening

Phytochemical analysis revealed the presence of coumarins, amino acids and tannins as major phytoconstitutes in methanolic fractions of leaves of *T.corniculata* while quinines, terpenoids, phenols, leucoanthocyanins were found to be absent altogether. Similar observations were reported earlier. Elnoor MEM and his coassociates in 2015[17] reported the presence of alkaloids, flavonoids, tannins, saponins, and terpenoids using seed and callus methanolic extracts using standard procedure. Premnath R *et. al,* 2011[22] reported that ethanolic extracts exhibited pronounced activity against *E.coli, P.aeruginosa, S.aureus* and other three bacterial species using leaf extracts of fenugreek.

Table 5 Phytochemical analysis for the methanolic extracts of *T.corniculata*

Extracts	Quinones	Terpenoids	Tanins	Phenols	Coumarins	Amino acids	Leucoanthocyanins
Methanol	-	-	+	+	+	+	-

They further estimated the total phenol (4.9mg/g) and flavonoid (0.47mg/g) content where phenolic content was found to be much higher and thus was explained as possible component for antimicrobial activity. Ethanolic extracts resulted in much higher concentration of phenols as compared to other solvent system. Moreover minimum inhibitory concentrations revealed the plant leaf ethanolic extracts are more effective against *S.aureus* followed by *P.aeruginosa*. The plant has been found to be a good source of bioactive compounds from different plant parts. Seeds consist of saponins (diosgenin and fenugreekine), Alkaloids (gentianine, carpaine) various amino acids and flavonoids. While phenolic compounds include coumarin, scopoletin, chlorogenic acid, caffeic acid and p-coumaric acid. Seeds also containd steroidal (choline) and piperidine (Carpaine) and pyridine (Trigonelline and gentianine) as described by Ritika, (2016)[24].

CONCLUSION

The present investigation is an insight for further elucidation of adulteration in market samples to original mother plant using molecular markers. The present results will be confirmed if any adulteration is responsible for the significant zones of

inhibition in market samples. Although the previous reports revealed that leaves extract are more potent against *B. subtilis* a gram positive bacteria but present investigation showed equivalent sensitivity of *E.coli* to plant leaf extracts resulting in significant zones of inhibition. The original mother plant was more effective compared to market samples which showed results dependency on particular solvent system. The plant needs to be further explored as is available easily and our research recommend to use original plant leaves in comparison to different market samples available for effective health care in present scenario where most of the food products are adulterated. The market samples were specifically soluble in petroleum ether solvent extracts and mostly active enough against *E. coli* a gram negative strain while previous report showed activity against *B. subtilis* a gram negative strain. The pharmaceutical companies and natural product manufacturers can cover up a large proportion of medicines and tablets by using kasuri methi as a substitute. Moreover the richness of iron content in kasuri methi makes it more potent plant product to be involved in daily intake to fight disease and improve immune system.

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