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Research Article

In Vitro Anti-Microbial and Anti-Fungal Activity of SuccessiveExtract of *G. Asiatica* Linn. Leaf.

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Abstract
Antibacterial activity of alcoholic extract of *G. asiatica* Linn leaves were comparable to standard (Streptomycin) and aqueous extract was less active than standard against Gm +ve (*B. subtilis*) organism. Antibacterial activity of aqueous extract was less than the standard (Ampicillin) against Gm –ve (*E. coli*) organism. Antifungal activity of aqueous extract was comparable to standard (Fluconazole) for S. cerevisae.

Introduction:

Synonyms: *Grewia subinaequalis* DC.

Biological Source[1]: Drug consists of dried whole plant of *Grewia asiatica* Linn. belonging to family Tiliaceae.

Part used: bark, fruits, leaves[2]

Plant of *Grewia asiatica* Linn.

Vernacular names[3]

Sanskrit : Dharmana, Parusha
Bengali : Shakri, Phalsa
English : Phalsa
Gujrati : Phalsa
Hindi : Phalsa
Malayalam : Sataschi
Marathi : Daman, Damni, Karavarani
Tamil : Tadachit, Sadachi,Una, Tarra
Telugu : Phutiki, Charachi, Ettatada, Nulijana
Punjabi : Phalna, Pharua

Description[2,3]

A shrub or small tree, young parts stellately pubescent.

- **Bark:** Rough and gray.
- **Leaves:** Leaves are 7-17/6-12 cm, ovate or suborbicular, acute or subacuminate or cuspidate, sharply and often coarsely doubly serrate, subglabrous above, hairy-tomentose beneath, rounded or only slightly cordate at the base 5-6-7 nerved; petioles 6-12 mm long, thickened at the top; stipules nearly as long as the petioles, linear, lanceolate.
- **Flower buds:** Flower-buds broadly cylindric or clavate. Peduncles axillary, usually many, long, slender, far exceeding the petioles and often 3-4 times as long, sometimes 4 cm long.
- **Flowers:** Flowers large. Bracts beneath the pedicels lanceolate. Sepals about 10 cm. long, linear oblong, acute, stellately pubescent or tomentose. Petals yellow, oblong or ovate-oblong, jagged or entire, about 6 mm. long, not bifid, gland with a wide fleshy margin, pubescent towards the edges. Gonophore long. Stigma with 4 short, rounded lobes; style much thickened above.
- **Fruit:** Fruit red, globose, 6-8 mm. diameter; pyrenes 1-2, always 1- celled only.

Habitat: Drier woodlands and on most soils as well as drier vine thicketsand coastal regions".

Materials And Method:[4,5,6,7]

Collection Of Plant Material

Aerial parts of *Grewia asiatica* Linn. herbs growing in natural habitat in Rampura, Panchmahal, Gujarat, India, were collected in June, 2018.

Micro-organisms used

Gram- Positive: *Bacillus subtilis* (ATCC6633)
Gram- Negative: *E. coli* (NCTC 6571)
Fungi: *Saccharomyces cerevisiae*

Preparation of Inoculums

Suspension of organism was prepared as per McFarland nephelometer standard (Ellen & Sydney 1990). A 24 hour old culture wasused for the preparation of bacterial suspension. Suspension of organism was made in a sterile isotonic solution of sodium chloride (0.9% w/v)

Procedure

The medium was prepared by dissolving all the ingredients in distilled water and subjected to sterilization in an autoclave at 121^0C for 15 minutes. The Petri plates were washed thoroughly and sterilized in hot air oven at 160^0C for 1 ½ hours. 30 ml of sterile molten agar medium was seeded by organisms (about 2 ml according to Mc Farland's standard), in semi hot conditions (40^0C) was poured aseptically in sterile Petri plate and allowed to solidify at room temperature. Bores were made on medium using sterile borer and 0.1 ml of extracts were added to respective bore and 0.1 ml of the standard at a concentration 0.5, 1.0 and 1.5 mg/ml was taken as standard. The Petri plates were incubated at 37^0C for 24 hours in a BOD incubator and zone of inhibition was observed and measured using a scale. The results of the antibacterial activity of *G. asiatica* Linn. Leaf are tabulated in Table 1,2,3,4.

Determination of zone of Inhibition

The extracts were prepared by successive extraction with petroleum ether, chloroform, alcohol and water of *G. asiatica* Linn. Leaf and these extracts were screened for their antibacterial and antifungal activity. All theextracts were dissolved in DMSO to get the concentration of 20, 40, 60 and 80 mg/ml. Evaluation of the activity was carried out by cup-plate technique using nutrient agar medium for bacteria and Sabourad's dextrose agar medium for fungus. Antibacterial & antifungal activity was measured in terms of zone of inhibition.

Pharmacological Evalution

Antibacterial activity of successive extract of *G. asiatica* Linn.leaves

Table 1 Antibacterial activity of Successive extracts of GA against (*B. subtilis*)

Concentration (mg/ml)	Diameter of zone of Inhibition in mm*			
	Pet. ether	Chloroform	Alcohol	Water
20	2.21 ± 0.12	1.20 ± 0.17	1.63 ± 0.15	1.45 ± 0.05
40	1.91 ± 0.68	1.22 ± 0.20	1.53 ± 0.15	1.85 ± 0.32
60	-	1.10 ± 0.10	1.83 ± 0.11	1.93 ± 0.40
80	-	1.27 ± 0.30	2.20 ± 0.10	1.90 ± 0.10

*values are in terms of Mean ± SEM of results done in triplicate

Alcohol and water extracts of *G. asiatica* Linn. leaves exhibited better antibacterial activity against gram positive organism (*Bacillus subtilis*) than their petroleum ether and chloroform extracts. Antibacterial activity may be due to Carbohydrates, Flavonoids, Steroids, Glycosides, and Tannins present in it.

Table 2: Antibacterial activity of Successive extract of GA against (*E. coli*)

Concentration (mg/ml)	Diameter of zone of Inhibition in mm*			
	Pet. ether	Chloroform	Alcohol	Water
20	-	1.32 ± 0.10	1.57 ± 0.60	1.97 ± 0.15
40	-	1.26 ± 0.15	1.53 ± 0.15	1.82 ± 0.20
60	-	1.30 ± 0.20	1.55 ± 0.13	2.03 ± 0.15
80	-	1.28 ± 0.19	1.65 ± 0.30	1.90 ± 0.17

*values are in terms of Mean ± SEM of results done in triplicate

Alcohol and water extract of *G. asiatica* Linn. leaves exhibited better antibacterial activity against gram negative organism (*E. coli*) as compared to their petroleum ether and chloroform extracts. Antibacterial activity may be due to Carbohydrates, Flavonoids, Steroids, Glycosides, and Tannins present in it.

Antifungal activity of successive extract of *G. asiatica* Linn.leaves

Table 3: Antifungal activity of Successive extract of GA against *S. cerevisae*

Concentration (mg/ml)	Diameter of zone of Inhibition in mm*			
	Pet. ether	Chloroform	Alcohol	Water
20	-	-	-	1.97 ± 0.15
40	-	-	-	2.00 ± 0.20
60	-	-	-	2.03 ± 0.15
80	-	-	-	2.08 ± 0.21

*values are in terms of Mean ± SEM of results done in triplicate

Water extract of *G. asiatica* Linn. leaves exhibited antifungal activity while petroleum ether, chloroform and alcoholic extracts did not shown antifungal activity against *Saccharomyces cerevisae*. Antifungal activity may be due to Carbohydrates, Flavonoids, Steroids, Glycosides, and Tannins present in it.

Anti-bacterial and Anti-fungal activity of standard drugs

Table 4: Anti-microbial and Anti-fungal activity of standards

Concentration	Diameter of zone of Inhibition in mm*

(mg/ml)	*B.subtilis*	*E.coli*	*S.cerevisae*
	Streptomycin	**Ampicillin**	**Fluconazole**
0.5	2.08 ± 0.076	2.76 ± 0.27	2.52 ± 0.16
1.0	2.11 ± 0.25	2.23 ± 0.49	2.17 ± 0.23
1.5	2.01 ± 0.12	2.40 ± 0.26	2.36 ±0.26
2.0	2.28 ±0.16	2.50 ± 0.79	2.22 ± 0.21

*values are in terms of Mean ± SEM of results done in triplicate

The result suggests that Antibacterial activity of alcoholic extract was comparable to standard (Streptomycin) and aqueous extract was less active than standard against Gm +ve (*B.subtilis*) organism. Antibacterial activity of aqueous extract was less than the standard (Ampicillin) against Gm –ve (*E.coli*) organism. Antifungal activity of aqueous extract was comparable to standard (Fluconazole) for *S. cerevisae*.

Antibacterial activity (gm +ve and gm -ve) and Anti fungal activity of Standard of *G. asiatica* Linn. S1, S2, S3

S_1 = Streptomycin; S_2 = Ampicillin; S_3= Fluconazole

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