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Evaluation of medicinal constituents and properties of *Linum usitatissimum*, *Prosopis juliflora* and *Guizotia abyssinica*

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Abstract

Linum usitatissimum, *Prosopis juliflora* and *Guizotia abyssinica* are remarkably important medicinal plants used for therapeutic purpose. The present study deals with phytochemical analysis of selected plants and also carried out to investigate antibacterial, antifungal and cytotoxic activity. The ethanolic extracts of seeds of the plants were tested for the presence of various phytochemical constituents such as tannins, terpenoids, alkaloids, flavanoids, phlobatannins, cardiac glycosides and steroids. The antibacterial activity was tested against human pathogenic micro-organisms like *Bacillus subtilis* (ATCC 6633), *Streptococcus aureus* (ATCC 6538), *Streptococcus abony* (ATCC 6017), *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 9027) by the agar well diffusion method. The ethanolic extracts of seeds of selected plants were tested against pathogenic fungi like *Alternaria solani*, *Aspergillus niger*, *Lasiodiplodia theobromae*, *Rhizopus spp.* and *Candida albicans* by pour plate method. The cytotoxic effect of selected plants were tested against HEK293T (Human embryonic kidney cell line) and c2c12 (Mouse, Muscle cell line) by MTT assay. Results showed that selected plants contain important phytochemicals which can be used to investigate its potential use for developing new drugs.

Keywords: *Linum usitatissimum*, *Prosopis juliflora*, *Guizotia abyssinica*, phytochemical, antibacterial, antifungal, cytotoxicity

Introduction

Since time immemorial, plants have provided mankind with a source of natural products of medicinal value (Balandrin *et al.*, 1993). Medicinal plants, which are the important constituents of traditional medicine need to be studied for their pharmacological value. Medicinal plants are a potential source of compounds of therapeutic value and can be exploited for the same.

Linum usitatissimum (Linaceae), (2n=30), commonly known as flax, is known for the production of one of the oldest commercial vegetable oil known as linseed oil (Diederichsen and Richards, 2003; Vaisey-Genser and Morris, 2003) [3, 16]. It has been reported to be effective in fighting prostate cancer (Zhao, G *et al.*, 2004; Zhao G *et al.*, 2007) [19, 18] breast cancer (Hutchins *et al.*, 2000) [7] and diabetes and reduces the risk of colon cancer by protecting colon cells from cancer causing toxins and free radicals.

Prosopis juliflora (Fabaceae), (2n=56), commonly known as mesquite or mostrenco contains piperidine, juliflorine, julifloricine, julifloridine, juliprosine, juliprosinene and juliflorinine with good antifungal, antimicrobial and antitumor activities (Singh, 2012) [11].

Guizotia abyssinica (Asteraceae), (2n = 30) is also known as noog/nug, Niger, nyger, nyjer or Niger seeds, ramtil or ramtilla; and blackseeds. It has good antifungal, antimicrobial, antitumor activities. Dutta *et al.*, (1994) [4] studied the lipid composition of six cultivars of Ethiopian Niger and found that the total lipid was triacylglycerides and polar lipids accounted for 0.7-0.8% of the total lipid content.

The present study aims at screening *L. usitatissimum*, *P. juliflora* and *G. abyssinica* for the presence of important phytochemicals, so that full pharmacological potential of the plant could be exploited. *B. subtilis*, *S. aureus*, *P. aeruginosa*, *E. coli*, *C. albicans*, *A. Niger*, *Alternaria*, and *Rhizopus* are most common plant pathogens and some are human pathogens as well. Hence, the study was undertaken for screening antimicrobial and antifungal activity of the plants against all these strains and some other strains like *S. abony* and *Lasiodiplodia*, which have not been well documented. The present work also aims at evaluating the cytotoxic activity of the selected plants using different concentrations of extracts against c2c12 (Mouse, Muscle cell line) and HEK293T (Human embryonic kidney cell line) using the MTT assay.

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Material and Methods

Procurement of plant material

Seeds of *Linum usitatissimum* and *Guizotia abyssinica* were procured from Nagpur. Seeds (pods) of *Prosopis juliflora* were procured from some arid regions of Kutch. Leaves and stem portion of *P. juliflora* were collected from a plant farm at Bedwa, Gujarat. The seed and leaves were air dried and ground well into a fine powder. The powder was stored at room temperature in air sealed polythene bags before extraction.

Preparation of plant extracts: (Tiwari *et al.*, 2011)^[14]

- Linum usitatissimum* extract:** Seeds were ground to a fine powder. 1kg of the powder was mixed with 2litres of 95% ethanol
- Prosopis juliflora* extract:** 1kg of the seed powder was added to 2litres of methanol.
- Guizotia abyssinica* extract:** 1kg of powdered seeds was mixed with 2litres of 80% ethanol.

All extracts were sonicated for 30 minutes. It was kept for 48 hours on a rotary shaker at room temperature. The extracts were filtered using Whatmann's filter paper no. 1 and used for the phytochemical analysis. The filtrate was poured in petridishes and kept for evaporation of the liquid solvents, after which, the crude extracts were dissolved in DMSO (Dimethyl sulfoxide) and used for antibacterial, antifungal and cytotoxic activity.

Phytochemical screening (Mariajancyrani, 2014; Tiwari *et al.*, 2012; Sofowara (1993); Trease and Evans (1989) and Harborne (1973)^[8, 14, 12, 15, 6]

The extracts were used to detect the presence of different phytochemical constituents:

- **Test for Tannins:** Few drops of FeCl₃ solution were added to filtrate. Appearance of brownish green or deep black blue colour indicated the presence of tannins.
- **Test for Terpenoids:** Filtrate was mixed with chloroform and concentrated H₂SO₄ was carefully added to form layer. A reddish brown colour or ring formed indicates the presence of terpenoids.
- **Test for Alkaloids:** Filtrate was treated with Hager's reagent (saturated picric acid solution) Formation of yellow colored precipitate indicates the presence of alkaloids.
- **Test for Flavonoids:**-Few drops of lead acetate solution were added in filtrate. Formation of yellow colour or precipitate indicates the presence of flavonoids.
- **Test for Phlobatannin:** Aqueous HCL was added in filtrate and boiled. Formation of red colour or precipitate indicates the presence of phlobatannins.
- **Test for Cardiac Glycoside:** Glacial acetic acid was added to filtrate followed by addition of few drops of FeCl₃ solution and concentrated H₂SO₄. Development of brown colour ring indicates the presence of cardiac glycosides.
- **Test for Steroids:** The filtrate was treated with few drops of concentrated H₂SO₄ and shaken. Formation of red colour indicates the presence of steroids.

Antimicrobial activity (Sukirtha and Growther, 2012)^[13]:

Ethanollic extracts of the seeds of *L. usitatissimum* and *G. abyssinica* and methanolic extract of *P. juliflora* leaves were prepared using soxhlet apparatus method. All the extracts were poured into sterile dry petriplates and the solvent was

evaporated. The sediments were scrapped off, dissolved in DMSO and used for testing antibacterial activity. The strains like *Bacillus subtilis* (ATCC 6633), *Streptococcus aureus* (ATCC 6538), *Streptococcus abony* (ATCC 6017), *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 9027) were used as test organisms against the selected plant extracts. The cultures were stored on nutrient agar slants at 4° C. The antimicrobial activity of the plant extracts was determined by the agar well diffusion method. Nutrient agar medium was poured into the sterilized plates and allowed to solidify. The inoculum of the microorganisms was spread on the medium. Wells were punctured and the extracts were poured into the well. Plates were incubated at 37°C for 24 hours. Antimicrobial activities were evaluated by measuring inhibition zone diameters.

Antifungal activity: (Shinde and Dhale, 2011; Vandita *et al.*, 2013; Monisha *et al.*, 2013)^[10, 17, 9]

Ethanollic extracts of all plants were tested against pathogenic fungi like *Alternaria solani*, *Lasiodiplodia theobromae*, *Rhizopus spp.* And *Aspergillus Niger*, causing infection in many plants. All these fungi were obtained from Anand Agriculture University and pure cultures were maintained on PDA. Fungal spores were grown in the potato dextrose broth on shaking condition for 1 to 2 days. In sufficient concentration, this broth was added to the autoclaved molten PDA. After proper mixing this medium was poured in sterile plates and allowed to solidify. Then wells were punctured and extracts were poured into the well. Plates were incubated in the incubator at 28°C for 24 to 48 hours. Antifungal activities were evaluated by measuring inhibition zone diameters.

Determination of cell viability

Preparation of plant extract: Ethanollic extract of seeds and leaves of *Linum usitatissimum*, and *Guizotia abyssinica* and methanolic extract of *Prosopis juliflora* were used for the study. All the extracts were poured into sterile dry petriplates and allowed to get evaporated. The sediments were scrapped off dissolved in DMSO and used for testing anticancer activity. Three concentrations, 50µg/ml, 250µg/ml, and 500µg/ml were prepared from that and used along with DMSO as a negative control for all the plants used in the study.

Cell-lines used: The cytotoxic activity of the extract was evaluated on c2c12 (Mouse, Muscle cell line) and HEK293T (Human embryonic kidney cell line) which were procured as a kind gift from Dr. C.G. Joshi, Anand Agriculture University, Anand. Both the cell lines were grown in DMEM supplemented with 10% foetal bovine serum (FBS) and 1% penicillin, streptomycin, neomycin (PSN) at 37°C in a 5% CO₂, 95% humidified atmosphere.

MTT cytotoxicity assay: (Bukhari and Dar, 2012)^[2] the principle of the test is to convert the yellow tetrazolium salt MTT to purple formazan crystals by metabolically active cells. The amount of formazan produced would be proportional to the number of viable cells. Cells were plated in 96-well flat bottom tissue culture plates at a density of approximately 10,000 cells/well and allowed to attach overnight at 37 °C. The cells were then incubated with the extract at different concentrations of 50 µg/mL, 250 µg/mL and 500 µg/mL for 24 hours. Untreated cultures and blank wells without cells received negative control for respective controls. Post drug exposure period, the cells were grown for

an additional 24 hours in extract-free fresh medium. Next, 20 µL of the MTT (5mg/ml) reagent was added to each well, and the plate was incubated for 4 hours at 37 °C. The MTT crystals were then solubilized in 200µl of DMSO. Absorbance measurements were made at 570 nm using a Biotek ELISA plate reader. Proliferation was expressed as the fraction of treated cells that survived in relation to untreated cultures. Each experiment included a set of negative controls (untreated cultures) and all experiments were

Performed in triplicate. The percentage of cytotoxicity was calculated using the following formula (Gacche and Pund, 2011)^[5]

$$\% \text{ Cytotoxicity} = (1 - \text{Abs test} / \text{Abs Control}) \times 100$$

Results
Phytochemical analysis

Table 1: Data on phytochemical constituents of *Linum usitatissimum*, *Prosopis juliflora* and *Guizotia abyssinica*.

Tests for.	<i>Linum usitatissimum</i>	<i>Prosopis juliflora</i>	<i>Guizotia Abyssinica</i>
Tannins	+ve	+ve	+ve
Terpenoids	+ve	+ve	+ve
Alkaloids	+ve	+ve	+ve
Flavonoids	+ve	+ve	+ve
Phlobatannins	-ve	-ve	-ve
Cardiac glycosides	+ve	+ve	+ve
Steroids	+ve	+ve	+ve

+ve=present, -ve = absent

The phytochemical screening of the plants showed the presence of tannins, terpenoids, alkaloids, flavonoids, cardiac glycosides and steroids

In respective extracts of seeds of *Linum usitatissimum*, *Prosopis juliflora*, *Guizotia abyssinica*. All extracts showed the absence of phlobatannins (Table-1).



L= *Linum usitatissimum*

P = *Prosopis juliflora*

N = *Guizotia abyssinica*

PC = phytochemical test, PC 1 = Tannin, PC 2 = terpenoids, PC 3 = alkaloids, PC 4 = flavonoids, PC 5 = phlobatannins, PC 6 = cardiac glycosides, PC 7 = steroids.

Antimicrobial activity

Table 2: Antibacterial activity of *Linum usitatissimum*, *Prosopis juliflora*, *Guizotia abyssinica*

Name of micro-organism	Zone of inhibition		
	<i>Linum usitatissimum</i>	<i>Prosopis juliflora</i>	<i>Guizotia abyssinica</i>
<i>B. subtilis</i>	6mm	24mm	12mm
<i>S. aureus</i>	10mm	20mm	8mm
<i>S. abony</i>	10mm	28mm	14mm
<i>E. coli</i>	14mm	26mm	14mm
<i>P. aeruginosa</i>	6mm	24mm	10mm

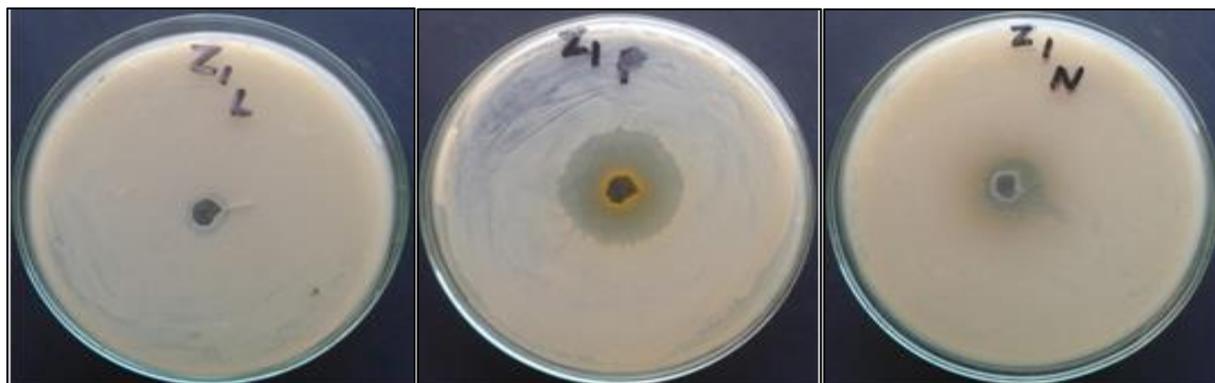


Fig 1: Zone of inhibition for *B. subtilis* 1.L=*Linum* 2.P=*Prosopis* 3.G=*Guizotia*

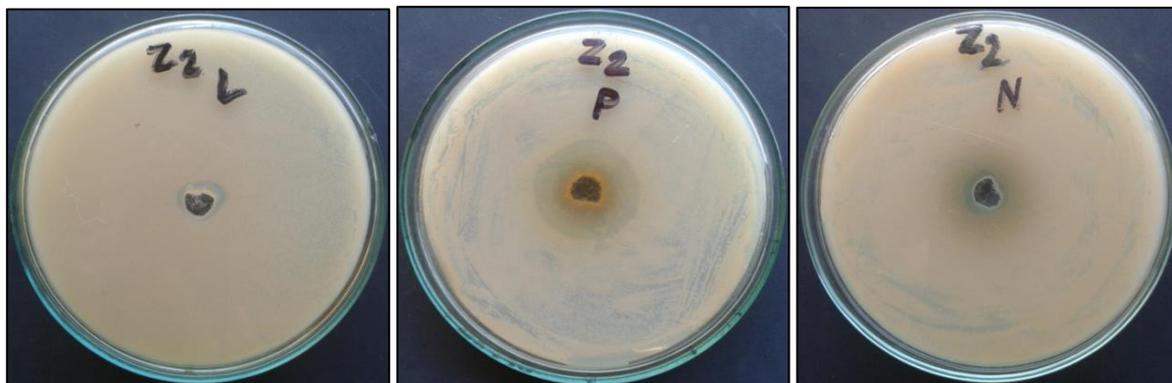


Fig 2: Zone of inhibition for *S. aureus* 1. *Linum* 2. *Prosopis* 3. *Guizotia*



Fig. 3: Zone of inhibition for *S. abony* 1. *Linum* 2. *Prosopis* 3. *Guizotia*



Fig 4: Zone of inhibition for *E. coli* 1. *Linum* 2. *Prosopis* 3. *Guizotia*



Fig 5: Zone of inhibition for *P. aeruginosa* 1. *Linum* 2. *Prosopis* 3. *Guizotia*

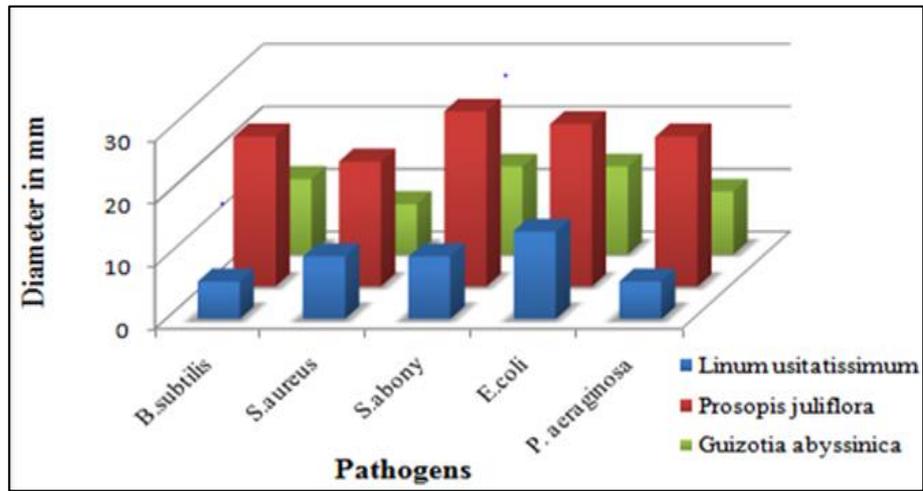


Fig 1: Inhibitory activity of defferent plant extracts against different bacteria

It was observed that the maximum inhibitory activity was shown by *Prosopis* extract against almost all micro-organisms studied. *Linum* and *Guizotia* also showed inhibition of microorganisms with higher activities against *E. coli*, *S. abony* and *B. subtilis*.

Extract of *Linum usitatissimum* was found to show highest zone of inhibition, 14mm against *E. coli*. *Prosopis juliflora* showed maximum inhibition against *S. abony* and *E. coli*. Maximum activity of *Guizotia abyssinica* was observed against *S. abony* and *E. coli*, followed by *B. subtilis* and *P. aeruginosa*. Amongst all the plants studied, *Prosopis juliflora* was having best activity against most of the pathogens tested compared to *Guizotia* and *Linum* (Graph-1).

Antifungal activity

Table 3: Antifungal activity of *Linum usitatissimum*, *Prosopis juliflora*, *Guizotia abyssinica*

Fungi	<i>Linum usitatissimum</i>	<i>Prosopis juliflora</i>	<i>Guizotia Abyssinica</i>
<i>Aspergillus niger</i>	16mm	42mm	74mm
<i>Alternaria solani</i>	-	28mm	-
<i>Lasiodiplodia theobromae</i>	30mm	44mm	16mm
<i>Rhizopus spp.</i>	20mm	44mm	16mm

L=*Linum usitatissimum*, P = *Prosopis juliflora*, N = *Guizotia abyssinica*,

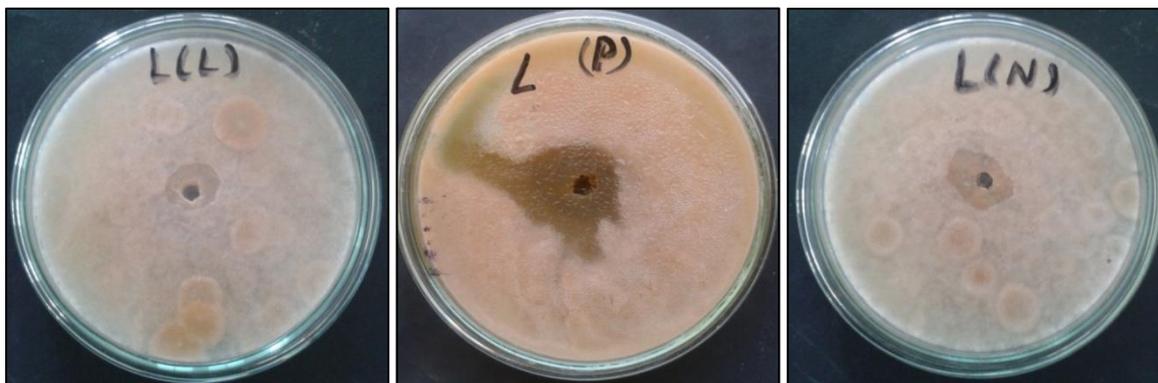
A.N = *Aspergillus Niger*, A = *Alternaria spp.*, L = *Lasiodiplodia theobromae*, R = *Rhizopus*



Fig 6: Zone of inhibition for *Aspergillus Niger*



Fig 7: Zone of inhibition for *Alternaria spp*

Fig 8: Zone of inhibition for *Lasiodiplodia theobromae*Fig 9: Zone of inhibition for *Rhizopus*

Linum extract was found to be most effective against *Lasiodiplodia*. However, it showed no inhibition against *Alternaria spp.* Extract of *Prosopis juliflora* was observed to show maximum inhibition against all the test strains. *Guizotia abyssinica* also reported good antifungal activity but it showed almost no inhibition to *Alternaria solani*. (Graph 2). Note: The plates containing *Linum* and *Guizotia* for activity against *Alternaria* were however discarded due to contamination.

Cytotoxic activity

The highest cytotoxic effect was found in the ethanolic extract of *Prosopis juliflora* seeds in 250 ug/ml concentration (73.63 ± 0.01) and methanolic extract of leaves (95.50 ± 0.01) against HEK293T in a concentration of 50 ug/ml. The ethanolic stem extract and methanolic leaf extract also showed considerable activity against c2c12 (245.81 ± 0.001 ; 95.50 ± 0.01) in 250 and 50 ug/ml respectively (Table 4). *Linum* and *Guizotia* also showed remarkable cytotoxic activities.

Table 4: Cytotoxic properties of *Linum usitatissimum*, *Prosopis juliflora* and *Guizotia abyssinica* on different cell line

Plant Name	Plant part	Solvent	Concentration (ug/ml)	% Cytotoxicity	
				HEK293T	c2c12
<i>Linum usitatissimum</i>	Seeds	Methanol	50	-3.63 ± 0.04	148.88 ± 0.010
			250	3.63 ± 0.22	211.73 ± 0.003
			500	77.27 ± 0.01	246.36 ± 0.004
<i>Prosopis juliflora</i>	Stem	Ethanol	50	20.90 ± 0.22	226.53 ± 0.011
			250	73.63 ± 0.01	245.81 ± 0.001
			500	62.72 ± 0.03	230.44 ± 0.006
	Leaves	Methanol	50	95.50 ± 0.01	262.56 ± 0.010
			250	88.76 ± 0.03	236.31 ± 0.009
			500	49.43 ± 0.08	203.07 ± 0.037
<i>Guizotia abyssinica</i>	Seeds	Ethanol	50	10.0 ± 0.05	119.55 ± 0.014
			250	10.90 ± 0.05	161.45 ± 0.011
			500	59.09 ± 0.11	113.12 ± 0.027

Results summarized here are the mean values from three parallel experiments \pm S.D.

NR = No reaction under experimental condition

Discussion

All the selected plants showed the presence of all phytochemical constituents including tannins, terpenoids, alkaloids, flavonoids, cardiac glycosides and steroids. Phlobatanins were however, absent in all the selected plants. *Prosopis juliflora* was remarkably effective against the

fungal and bacterial strains studied. In the cytotoxicity assay, *Prosopis* performed better than *Linum* and *Guizotia*.

Conclusions

From the above observations, it can be concluded that *Prosopis juliflora* has significantly high medicinal value compared to *Linum usitatissimum* and *Guizotia abyssinica* in

terms of phytochemical, antimicrobial, antifungal and anticancer activities. Hence these plants can be exploited for developing therapy against different bacterial and fungal diseases. These plants can also be exploited as a potential anticancer agent.

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