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Original Research Article

Evaluation of Phytochemical and Antimicrobial Potential of Endophytic Fungi Nigrospora Oryzae Isolated from Terminalia Bellerica Roxb.

Jagadevi Shivaputrappa[1], Vidyasagar GM[2]

[1]Research Scholar, [2]Professor, Medicinal Plants and Microbiology Research Laboratory, Department of Post Graduate Studies and Research in Botany, Gulbarga University, Kalaburagi- 585106, Karnataka, India.

Corresponding Author: Vidyasagar GM

ABSTRACT

Microorganisms are divided in to two categories; they are beneficial and harmful microorganisms. Beneficial microorganisms helps human beings where as harmful microorganisms causes several dreadful diseases to human beings. There are several antimicrobial drugs are available but the microbes became resistant to available drugs. So, there is an urgent need to develop a new therapeutic agent with least side effects. Terminalia bellerica is an important ethnomedicinal plant and this is the first report of endophytic fungi from this plant.

Objective: To evaluate the phytochemical and antimicrobial activity of an endophytic fungi Nigrospora oryzae.

Materials and methods: The phytochemical analysis of all the extracts of fungi was carried out by referring the standard procedures and antimicrobial activity was carried out by agar well diffusion method.

Results: The phytochemical investigation of all the extracts of fungi showed the presence of alkaloids, phenols, flavonoids, tannins, saponins, steroids, triterpenes and glycosides. The methanol, ethanol and ethyl acetate extracts of endophytic fungi were tested against five human pathogenic Bacteria like, Klebsiella pneumoniae, Salmonella typhi, Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli and four Candida species like, C. albicans [MTCC 1637], C. glabrata [MTCC 3019], C. haemulonii [MTCC 1966] and C. tropicalis [MTCC 230] by agar well diffusion method. The methanolic and alcoholic extract of N. oryzae at 40mg/ml showed maximum activity against S. aureus (17mm), S. typhi (18mm), C. Tropicalis (14mm) and C. albicans (15mm), respectively.

Conclusion: N. oryzae *possess* several bioactive molecules which are responsible for antimicrobial potential, may serve as an alternative source for the treatment and control of several microbial infections.

Key Words: Terminalia bellerica, Nigrospora oryzae, Stem bark, endophytic fungi

INTRODUCTION

At present microbial infections are the major reason for the human illness, to cure these there are several drugs are available in the market but the microbes became resistant to available drugs. There is an urgent need for the hunting of new therapeutic compounds; the scientific community is moving towards the alternate, safe and cost effective sources of medicine. Medicinal plants are the unique source of potential bioactive molecules since ancient times. In traditional medicine they use large amount of plants for the treatment of

diseases. The over use of a particular plant species leads to inclusion of its name in red data book [1] and it is an alarming sign for scientific community to protect the plant population. So the scientists turned towards endophytic fungi, are the warehouse of several potential drugs. Recent reports had shown that fungal endophytes could also produce metabolites similar to or with more activity than their host plant. [2] Since the microbial sources of bioactive compounds are easier and more economical for large-scale production than plant sources. [3] Endophytic fungi are the gold mines of plant Kingdom, in future it is the only such source that can fulfil the needs of human health problems. India is the poor country but it has rich biodiversity that is not there in any other country in the world. So, it is the boon for scientists in their research to isolate several bioactive molecules, which helps in curing many dreadful diseases of human population. *Terminalia bellerica*, the versatile traditional medicinal plant belongs to the Combretaceae family, is the rich source of bioactive compounds with diverse chemical structure. The various aerial parts of *T. bellerica* has been screened for phytochemical and pharmacological properties such as hepatoprotective, [4-7] Purgative, [8] Choleretic, [9] hypotensive, [10] antispasmodic, antiasthmatic, antitussive [11] anti-mutagenic, [12] antimicrobial, anti-HIV-1 [13] anti-atherosclerosis, [14-16] anti-inflammatory, antioxidant [17] and anti diabetic [18] and there is no reports on endophytic fungi of this plant. Keeping in view of medicinal importance of *Terminalia bellerica*, endophytic fungi *Nigrospora oryzae* was isolated from the stem bark and its phytochemical and antimicrobial activity was carried out to evaluate the biochemical constituents and its effect on the disease causing microorganisms.

MATERIALS AND METHODS

Collection of plant material

Stem bark of *T. bellerica* was collected from the Botanical Garden of Gulbarga University, Kalaburagi, Karnataka, India. The plant was authenticated with the help of Flora of Gulbarga district. A specimen was deposited in the herbarium, Department of Botany, Gulbarga University, Kalaburagi, Karnataka, India with voucher specimen number HGUG- 141. [19] The stem barks were cut with the help of a sterile scalpel and placed in sterile plastic bag, then brought to the laboratory and processed within 24 h of collection.

Isolation and identification of endophytic fungi

Sample was washed thoroughly in running tap water before processing. Stem bark was surface sterilized by sequential washes in 70% (v/v) ethanol (1 min) and 3.5% (v/v) NaOCl (2 min), rinsed with sterile water and allowed to surface dry under sterile conditions. The sterile plant material were cut in to small segments (0.5 X 0.5 cm) and placed on PDA medium which is previously poured in sterile petriplates, supplemented with streptomycin and ketoconazole (100 µg/ml). Three segments were placed on PDA medium in each Petri dish. The Petri dishes were sealed using Para film and incubated in a light chamber for 2 weeks at 12 h light and dark cycles at 23^0C. [20] After incubation for 15 or more days, fungal colonies was observed, then individual fungal colonies were picked from the edge with a sterile fine tipped needle and transferred onto PDA and is maintained as pure culture. The fungi were identified based on fungal culture morphology (fig-1) and conidial characters (fig-2) and by using the available literature. [21,22]

Preparation of crude extract

The isolate is initially cultured on PDA and incubated as described previously. Cultured fungal fragments were obtained from the actively growing margin of the fungal colony culture and inoculated in to 500 ml conical flasks containing 300 ml PD broth (pH 5.8). The inoculated broth was incubated at 21±2°C for a period of 8-10 days kept on shaker for mass cultivation. The culture broth was filtered through three

layered muslin cloth to separate out the mycelial mat from the culture filtrate. The mycelial mat was washed with double distilled water to remove the broth content and ground in a pestle and mortar using ethanol, methanol and ethyl acetate separately. The grounded mycelia was then transferred into three different conical flasks containing ethanol, methanol and ethyl acetate, kept shaking for 3-4 days and filtered with cheese cloth. The filtrate was collected and evaporated to dryness. The extract residue was dissolved in dimethyl sulfoxide (DMSO) and stored at 4°C to be used as stock solution for determining the phytochemical and antimicrobial activity.

Phytochemical screening

The preliminary phytochemical studies were performed for the evolution of different chemical groups present in methanol, alcohol and ethyl acetate extracts of *Nigrospora oryzae*.

1. Test for Alkaloids

a. Dragendorff's test- To 2 mg of the extract 5 ml of distilled water was added; 2ml Hydrochloric acid was added until an acid reaction occurs. To this 1 ml of Dragendorff's reagent extract was added. Formation of orange or orange red precipitate indicated the presence of alkaloids.
b. Mayer's test- To 2 mg of the extract taken in a test tube, a few drops of Mayer's reagent was added. Formation of a yellow / white precipitate confirmed the presence of alkaloids.
c. Wagner's test- 2 mg of the extract was acidified with 1.5 % v/v of hydrochloric acid and a few drops of Wagner's reagent were added. A yellow or brown precipitate indicated the presence of alkaloids. [23]

2. Test for Phenols

a. Ellagic Acid test - The test solution was treated with a few drops of 5% (w/v) glacial acetic acid and 5% (w/v) NaNO2 solution. The solution turned muddy or Niger brown precipitate occurred in the extract. It indicates the presence of phenol solution.
b. Ferric chloride test - 0.5 ml of FeCl3 (w/v) solution was added in 2 ml of test solution, formation of an intense color indicates the presence of phenols. [24]

3. Test for Flavonoids

a. Shinoda's test - In a test tube containing 0.5 ml of the extract 10 drops of dilute hydrochloric acid followed by a small piece of magnesium were added. Formation of pink, reddish or brown colour indicated the presence of flavonoids.
b. Ferric chloride test - Test solution with a few drops of ferric chloride solution shows intense green colour.
c. Zinc-Hydrochloric acid reduction test - Test solution with zinc dust and a few drops of hydrochloric acid shows magenta red colour.
d. Alkaline reagent test - Test solution when treated with sodium hydroxide solution, shows an increase in the intensity of yellow colour which becomes colourless on addition of a few drops of dilute acid.
e. Lead acetate solution test - Test solution with a few drops of lead acetate (10%) solution gives a yellow precipitate.

4. Test for Triterpenoids

a. Liebermann - Burchard's test (LB test) - 2 mg of dry extract was dissolved in acetic anhydride, heated to boiling, cooled and then 1 ml of concentrated sulphuric acid was added along the sides of the test tube. Formation of a violet coloured ring indicated the presence of triterpenoids.
b. Salkowaski test - When a few drops of concentrated sulphuric acid were added to the test solution, shaken and allowed to stand, lower layer turns yellow indicating the presence of triterpenoids.

5. Test for Saponins

a. Foam test - In a test tube containing about 5 ml of extract, a drop of sodium bicarbonate solution was added. The test tube was shaken vigorously and left for 3 minutes. Formation of honeycomb like froth indicated the presence of saponins.

6. Test for Steroids

a. Liebermann-Burchard's test - 2 mg of dry extract was dissolved in acetic anhydride, heated to boiling, cooled and then 1 ml of concentrated sulphuric acid was added along the sides of the test tube. Formation of green colour indicated the presence of steroids.
b. Salkowaski reaction - 2 mg of dry extract was shaken with chloroform, to the chloroform layer sulphuric acid was added slowly by the sides of the test tube. Formation of red colour indicated the presence of steroids.

7. Test for Tannins

a. Ferric chloride test - To 1-2 ml of the extract, few drops of 5% w/v FeCl3 solution were added. A green colour indicated the presence of gallotannins, while brown colour indicated the presence of pseudotannins.
b. Gelatin test - Test solution when treated with a gelatin solution gives white precipitate colour. This confirmed the presence of a naphthoquinone. [25]

8. Test for glycosides

a. Baljet test -The test solution was treated with sodium picrate gives orange colour
b. Keller-Killiani test - The test solution was treated with a few drops of ferric chloride solution and mixed. When concentrated sulphuric acid containing ferric chloride solution was added, it forms two layers, lower layer reddish brown and upper acetic acid layer turns bluish green.
c. Raymond's test - Test solution when treated with dinitro- benzene in hot methanolic alkali, gives violet colour.
d. Bromine water test -Test solution when treated with bromine water gives yellow precipitate.
e. Legal's test - Test solution when treated with pyridine (made alkaline by adding sodium nitroprusside solution) gives pink to red colour.

Source of Test Microorganisms

Antimicrobial activity was carried out using the ethanol, methanol and ethyl acetate crude extracts of *N.oryzae* against five bacteria and four Candida strains using agar well diffusion method. Bacteria strains like *S.aureus, E.coli, S. typhi, P. aeruginosa* and *K. pneumoniae* and four candida species like *Candida albicans, Candida glabrata, Candida haemulonii* and *Candida tropicalis* were used for antimicrobial activity. The above mentioned bacteria strains were obtained from the Medicinal plants and Microbiology laboratory, Department of Botany, Gulbarga University, Kalaburagi, Karnataka, India., and Candida species were taken from microbial type culture collection (MTCC) Chandigarh, India.

Preparation of sample

For the preparation of test sample, 40mg of crude extract of *N.oryzae* was dissolved in Dimethyl sulphoxide (DMSO). The corresponding concentration was expressed in terms of mg of extract per ml of solvent (mg/ml).

Antimicrobial activity

The crude extract from the endophyte *N. Oryzae* were tested against five human pathogenic bacteria like *S. typhi, S. aureus, K. Pneumonia, P. aeruginosa* and *E. coli* and four *candida* species like *C. albicans, C. glabrata, C. haemulonii* and *C. tropicalis* using a concentration of 100µl as inoculum for antimicrobial activity. The antimicrobial activity was carried out by agar well diffusion method. Nutrient agar and YPD plates were inoculated with an overnight culture of each bacteria and candida suspension. The inoculated organisms were evenly spread out using sterile cotton swabs. The wells were bored with 6mm cork borer and wells were poured with 40, 30, 20, and 10 mg/mL concentration of the sample. In other wells, supplements of DMSO was used as negative control and reference antimicrobial drugs Streptomycin for bacteria and Ketoconazole (100µg/ml) for fungi were used as positive controls, respectively. The experiment was carried out in triplicate. The plates were

incubated at 32ºC for 24h and results were recorded as zone of inhibition in mm.

RESULTS

The fig-a & b, is mycelial morphology of endophytic fungi arising from plant segment and microscopic image of fungal conidia. Phytochemical screening of crude extracts of the endophyte *N. oryzae* isolated from the stem bark of *T.bellerica* was done for the presence of various bioactive molecules. Alcohol extract contains alkaloids, flavonoids, steroids, phenols, saponins, terpinoids, tannins and glycosides and methanol extract contains all the biomolecules except tannins and saponins, whereas in ethyl acetate extract except saponins, steroids and tannins, all the components are present which is shown in table-1. The antibacterial and anticandida activity of various extracts of *N. oryzae* and its comparison with reference drugs is showed in table 2. Among five bacteria and four candida strains tested, *S.aureus* (17mm), *S.typhi* (18mm), *C.tropicalis* (14mm) and *C.albicans* (15mm) showed maximum zone of inhibition to methanol and alcoholic extract of *N. oryzae* respectively. The inhibition zones were nearly as comparable to reference drugs (Streptomycin and Ketoconazole). The results shows that bioactive principles having antibacterial and anticandida activity are present in the culture filtrate of the endophytic fungi.

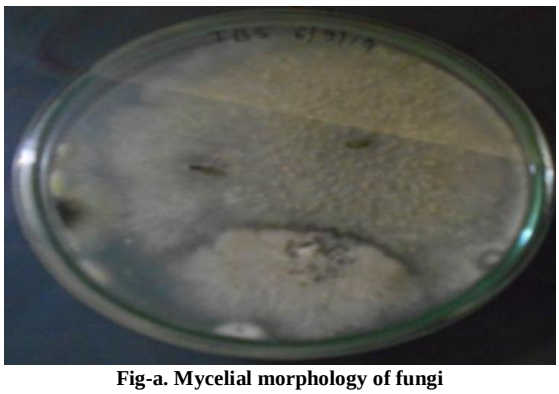

Fig-a. Mycelial morphology of fungi

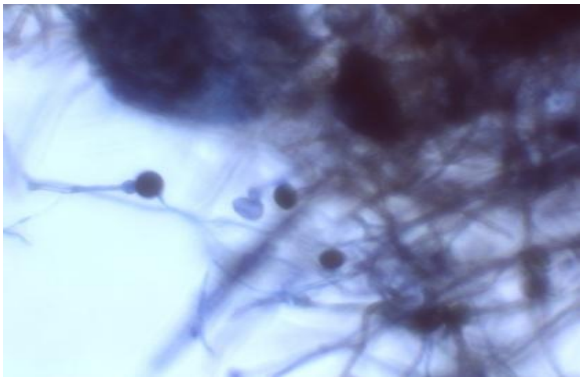

Fig-b. Microscopic image of fungi

Table-1. Phytochemical Screening of different extracts of endophytic *N.oryzae* isolated from stem bark of *T. bellerica.*

Phytochemical Tests	Methanolic extract	Ethanolic extract	Ethyl acetate extract
Alkaloids			
Dragendorff's test	-	-	+
Wagner's test	-	-	+
Mayer's test	+	+	-
Flavonoids			
Shinoda test	-	-	+
Ferric chloride test	+	+	+
Zinc Hydrochloric acid reduction test	-	-	-
Alkaline reagent test	+	+	-
Lead acetate test	+	+	+
Phenols			
Feric chloride test	+	+	+
Ellagic acid test	-	-	+
Terpinoids			
Liebermann Burchard's test	+	+	+
Salkowaski test	+	+	+
Saponins			
Foam test	-	+	-
Steroids			
Liebermann - Burchard's test	+	+	-
Salkowaski test	-	-	-
Tannins			
Ferric chloride test	-	+	-
Gelatin test	-	-	-
Glycosides			
Baljet test	-	-	-
Keller-Killiani test	+	+	+
Raymond's test	-	-	-
Bromine water test	+	+	-
Legal's test	-	-	+

+ Presence of compound; - Absence of compound.

Table-2. Antimicrobial efficacy of different extracts of endophytic *N. oryzae* isolated from stem bark of *T. bellerica*.

Test Organisms	Zone of Inhibition (mm) in different solvent system												Standards (100µg/ml)
	Ethylacetate extract				Methanolic extract				Alcoholic extract				Streptomycin/ Ketoconazole
	40	30	20	10	40	30	20	10	40	30	20	10	
S. aureus	9	7	6	5	17	15	13	12	12	10	7	6	18
K. pneumoniae	10	9	7	5	11	10	9	7	8	7	6	5	14
S. typhi	11	10	7	6	14	13	10	8	18	16	12	9	20
E. coli	9	7	5	-	10	8	7	5	11	10	8	5	13
P. aeruginosa	8	6	5	-	9	7	6	4	12	10	7	5	15
C. glabrata	12	10	7	4	10	8	7	-	11	9	7	-	18
C. tropicalis	10	9	7	5	14	12	10	8	12	10	8	5	13
C. haemulonii	6	4	4	-	6	5	3	-	7	6	4	3	11
C. albicans	8	6	6	5	8	7	6	3	15	14	12	10	16

The methanolic and alcoholic extracts of *N.oryzae* at 40µg/ml showed maximum activity. Against *S.aureus* (17mm), *S.typhi* (18mm) *C.Tropicalis* (14mm) and *C.albicans* (15mm) respectively

DISCUSSION

Medicinal plants are the warehouse of several potential bioactive molecules and are well known for curing several human ailments since ancient times. The available literature describes about the efficacy of *T. bellerica* leaf, fruit and stem bark extracts on many human pathogenic microorganisms. There are several reports available on fruit and leaf but very few on its stem bark. The methanolic extract of fruit showed maximum zone of inhibition against *S.aureus.* [26] The hexane and acetone extracts of leaf and stem is screened for antimicrobial activity using five gram positive bacteria like *Bacillus cereus, Bacillus subtilis, Listeria monocytogens, corynebacterium rubrum* and *staphylococcus epidermidis,* five gram negative bacteria such as *Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumonia. Escherichia coli* and *Salmonella typhimurium* and three fungal strains like *Candida albicans, Candida glabrata and Cryptococcus neoformans.* Among these the gram positive bacteria like *corynebacterium rubrum* and *staphylococcus epidermidis* and gram negative bacteria like *Klebsiella pneumonia. Escherichia coli* and *Salmonella typhimurium* showed very good results but no zone of inhibition against fungi. [27] The aqueous, petroleum ether and chloroform extracts of *Terminalia bellerica* fruits was used for phytochemical and antimicrobial activity. The phytochemicals like alkaloids, phenols, flavonoids and tannins are present in all the solvent extracts and the antimicrobial activity of water extract showed very good results against bacterial strains like *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Shigella flexneri,* and *Salmonella typhi* and fungal strains like *Aspergillus niger, Mucor species, Aspergillus fumigatus, Rhizopus species* and *Aspergillus flavus* and the remaining solvent extracts showed moderate activity. [28] The methanolic extract of *T.bellerica* was tested against two respiratory pathogens like *S.aureus* and *K. pneumoniae* showed significant activity [29] The antimicrobial activity of fruits of *T.bellerica* was carried out using hexane, benzene, chloroform, Ethyl acetate, Acetone, Ethyl alcohol and methanolic extracts against four bacteria and two viruses, among all the extract tested methanol and ethyl acetate extracts gave good results against NDV and PV virus and *S. aureus* and *E. Coli.* [30] As compared to previous reports, the present study proves that the methanol and alcoholic extracts of this fungus could be a possible source of new therapeutic agent against various infectious diseases. Hence the endophytic fungi *N.oryzae* isolated from stem bark of *T.bellerica* have ability to produce several bioactive molecules which will be useful in curing several human health problems.

CONCLUSION

It is interesting to note that crude extracts of *N.oryzae* possess several bioactive components which are responsible for antimicrobial potential, which might be

further explored to be used as an alternative source for the treatment and control of some microbial infections. Further investigations are needed to develop a standard anti-microbial drug.

REFERENCES

1. Ahmedullah M and Nayar MP. Red data book of Indian plants (Peninsular India). Vol. 4. Calcutta: Botanical Survey of India; 1999.
2. Storbel GA. Rainforest Endophytes and Bioactive Products. Crit Rev Biotechnology. 2002; 22: 315.
3. Storbel G and Daisy B. Bioprospecting for microbial endophytes and their natural products. Microbiol Mol Biol Rev. 2003; 67: 491-502.
4. Nadakarni AK. Dr. K.M. Nadakarnis Indian Materia Medica. 3rd Edn. Bombay, India: popular Book Depot; 1954. pp: 1202.
5. Anand KK, Singh B, Saxena AK, Chandan BK and Gupta VN. Hepatoprotective studies of a fraction from the fruits of Terminalia bellerica Roxb. On experimental liver injury in rodents. Phytother Res. 1994; 8: 287-292.
6. Anand KK, Singh B, Saxena AK, Chandan BK, Gupta VN and Bhardwaj V. 3,4,5-Trihydroxy benzoic acid (gallic acid) the hepatoprotective principle in the fruits of Terminalia bellerica – bioassay guided activity. Pharmacol Res. 1997; 36: 315-321.
7. Anjana J, Monika B and Sangeeta S. Protective effect of Terminalia bellerica Roxb. And gallic acid against carbon tetrachloride induced damage in albino rats. J Ethnopharmacol. 2007; 109: 214-218.
8. Chakravarti MD and Tayal JN. Preliminary examination of the fruits of Terminalia bellerica Roxb. Sci Cult. 1947; 13: 122-126.
9. Siddiqui HN. Studies on Terminalia bellerica Roxb. Effect on bile secretion and pharmacodynamic properties. Indian J Pharmacol. 1963; 25: 297-302.
10. Srivastava RD, Dwivedi S, Sreenivasan KK and Chandrashekhar CN. Cardiovascular effects of Terminalia species of plants. Indian Drugs. 1992; 29: 144-149.
11. Trivedi VP, Nesamany S and Sharma VK. A clinical study of the anti-tussive and anti-asthamatic effects of Vibhitakphal Churna (Terminalia bellerica Roxb.) in the cases of Kasa-Swasa. J Res Ayurveda Siddha. 1979; 3: 1-8.
12. Kaur S, Arora S, Kaur K and Kumar S. The in vitro antimutagenic activity of Triphala, an Indian herbal drug. Food Chem Toxicol. 2002; 40: 527-534.
13. Valsaraj R, Pushpangadan P, Smitt UW, Adsersen A and Christensen SB. New anti-HIV-1, antimalarial and antifungal compounds from Terminalia bellerica. J Nat Prod. 1997; 60: 739-742.
14. Tariq M, Hussain SJ, Saif M and Jahan M. Protective effect of fruit extracts of Emblica officinalis (Gaertn) Terminalia bellerica (Roxb.) in experimental myocardial necrosis in rats. Indian J Exp Biol. 1997; 15: 485-486.
15. Thakur CP, Thakur B, Singh S, Sinha PK and Sinha SK. The ayurvedic medicines Haritaki, Amala and Bahira reduce cholesterol induced atherosclerosis in rabbits. Int J Cardiol. 1988; 21: 167-175.
16. Shaila HP, Udupa AL and Udupa SL. Preventive actions of Terminalia Bellerica in experimentally induced atherosclerosis. Int J Cardiol. 1995; 49: 101-106.
17. Dash SK and Padhy S. Review on ethnomedicines for diarrhea diseases from Orissa Prevalence versus culthre. J Hum Ecol. 2006; 20: 59 -64.
18. Sabu MC and Kuttan R. Anti-diabetic activity of medicinal plants and its relationship with their antioxidant property. J Ethanopharmacol. 2002; 81: 155-160.
19. Seetharam YN, Kotresha K and Uplaonkar SB. Flora of Gulbarga District. First edition. 2000.

20. Suryanarayanan TS. Light-incubation: a neglected procedure in mycology. The Mycologist. 1992; 6: 144.
21. Nagamani A, Kunwar IK and Manoharachary C. Hand book of Soil fungi. New Delhi: I. K. International Pvt. Ltd; 2006.
22. Barnett HL and Hunter BB. Illustrated genera of imperfect fungi. 3rd Edn. Burgess Pub. Co; 1972.
23. Saldanha CJ. Flora of Karnataka. vol.1. New Delhi: Oxford and IBH Publishing Co; 1984.
24. Memelink J, Verpoort R and Kigine JW. Organisation of jasmonate responsive gene expression in alkaloid metabolism. Trends in plant sci. 2001; 6 (5):212-9.
25. Gibbs RD. Chemotaxonomy of Flowering Plants. Vol.1. London: McGill Queen's University Press, Montreal; 1974.
26. Elizabeth KM. Antimicrobial activity of Terminalia bellerica. Indian Journal of Clinical Biochemistry. 2005; 20 (2): 150-153.
27. Sumitra Chanda, Disha Menpara and Dishant Desai, Antimicrobial activity of Terminalia bellerica leaf and stem collected from two different sites. American Journal of Phytomedicine and Clinical Therapeutics. 2013; 1(9): 721-733.
28. Nithya devi P, Kaleeswari S and Poonkothai M. Antimicrobial activity and Phytochemical analysis of Terminalia. International Journal of Pharmacy and Pharmaceutical Sciences. 2014; 6 (5).
29. Shilpa Sabnis. Antimicrobial efficacy of Terminalia bellerica against virulence factors of respiratory pathogens. Int J Curr Microbiol App Sci. 2014; 3(5): 215-221.
30. Girija Nagendraswamy, Lakshmi Ranganatha V, Bushra Begum A, Prashanth T, Mohammed Alghorbani, Farhan Zameer, Shaukath Ara Khanum. Extraction and Evaluation of Antimicrobial and Antiviral Efficacy of *Terminalia bellirica* Fruits. Indo American Journal of Pharmaceutical Research. 2013; 3 (2).

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