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TERMINALIA BELLERICA – A PROMISING CHALLENGE TO CRYPTOCOCCOSIS

VALLI S[1], S GOKUL SHANKAR [2]

1. Research Scholar, Department of Biotechnology Acharya Nagarjuna University, Guntur, Andhra Pradesh& Assistant Professor in Microbiology, Mohamed Sathak college, Chennai.
2. Research Guide Department of Biotechnology Acharya Nagarjuna University, Guntur, Andhra Pradesh &Unit Of Microbiology, Faculty of Medicine, AIMST University, Malaysia.

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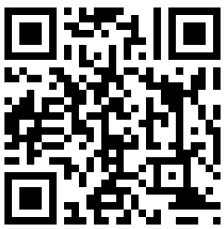
Abstract: **Background**- The increasing prevalence of multidrug resistant strains of fungi and the recent emergence of strains with reduced susceptibility to antifungal agents increases the specter of untreatable fungal infections and adds urgency to the search for new infection-fighting strategies which involves the utilization of bioactive phytochemicals from plants. **Objectives** :To study the antifungal activity of fruit of ***Terminalia bellerica*** against clinical and environmental isolates of ***Cryptococcus neoformans*** and to identify the bioactive compounds by GC MS FTIR. **Materials &Methods**: The ethanolic extract obtained from the fruit of ***Terminalia bellerica*** was assessed for their antifungal activity against clinical and environmental isolates of ***C neoformans***. Extracts were prepared from the fruits by standard techniques and phytochemical analysis of the extract was performed to study the bioactive compounds The compounds were identified by GC MS .analysis and the functional groups were identified by FTIR. **Result:** Of the 10 isolates tested the clinical isolates were more susceptible than the environmental isolates. C3 and E3 were found to be more susceptible with the zone of inhibition 18.5and 18mmrespectively at a concentration of 4mg/ml.. Mass spectrometric analysis revealed the presence of phenolic compounds and tannins .FTIR analysis revealed the presence of alkanes, alkynes, esters, carboxylic acids etc. as chemical groups constituting the bioactive compounds**. Conclusion** : Extract from fruits of ***Terminalia bellerica*** was found to possess antifungal activity and have the potential to inhibit drug resistant fungal strains .GC MS analysis revealed that leaves of ***Terminalia bellerica*** are rich source of tannins and polyphenolic compounds

Keywords: ***Terminalia bellerica,*** antifungal, pyrogallol, benzenetriol , GC MS FTIR



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Corresponding Author: Mrs. VALLI S.

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INTRODUCTION

The last two decades have faced a dramatic rise in the incidence of life threatening systemic fungal infections. The challenge has been to develop effective strategies for the treatment of fungal diseases, considering the increase in opportunistic fungal infections in HIV positive patients and in others who are immunocompromised .The emergence of antibiotic resistance in microbes due to indiscriminate use of chemicals as antifungals requires the need to look for alternative sources of antimicrobial agents One of the possible strategies towards this objective involves use of bioactive phytochemicals having antifungal activity

There has been increasing interest in alternative therapies and therapeutic use of natural products derived from plants .This interest in plant based drugs may be due to side effects of conventional medicine and ecological awareness that natural products are harmless. However the potential use of higher plants as drugs is still poorly explored. Pharmacological screening and phytochemical investigation has been carried out only in very few plants. It is estimated that 5000 sp have been studied for medical use [1] The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents have lead to the screening of several medicinal plants for their potential antimicrobial activity

Terminalia bellerica Roxb. also referred to as, Beleric Myrobalan belonging to family Combretaceae is a large deciduous tree with a thick brownish gray bark with shallow longitudinal fissures, attaining a height of between 20and 30 meters. [2] The fruits are ovoid grey drupes, 2.5 to 4cm in diameter, mature fruits with slightly wrinkled appearance. It is used as antidiabetic, laxative, anticancer and antimicrobial. It has antioxidant activity and hepatoprotective activity It is routinely used as traditional medicine, to get remedies from several ailments such as fever, cough, diarrhea, skin diseases and oral thrush. Chemical substances of β-sitosterol, gallic acid, ethyle gallate, galloyl glucose, a new triterpene, the belleric acid and chebulagic acid have been reported from fruits of *T .bellerica.*

Due to the medicinal value of ***Terminalia bellerica*** the present work was carried out to examine the antifungal potential of crude extracts of the fruits against clinical and environmental isolates of ***Cryptococcus neoformans***

MATERIALS AND METHODS:

Preparation of Extract:

The fruits of ***T bellerica*** were purchased commercially From dried fruits seeds were separated and the remaining part was made into coarse powder .The coarse powder was then extracted with ethanol by Soxhlet apparatus. The extract was filtered

using Whatmann filter paper and then concentrated in vacuum and air dried.

Anticryptococcal activity of ethanolic fruit extract of *Terminalia bellerica:*

The anticryptococcal activity of crude alkaloids was evaluated by disc diffusion method of NCCLS National Committee for clinical Laboratory Standards[3] Activated cultures of 10 isolates of C neoformans in Sabouraud Dextrose broth were adjusted to $1x10^8$ CFU /ml as per McFarland standard. 100µl of the inoculum was introduced to molten Sabouraud dextrose agar and poured into sterile petriplates

Sterile filter paper discs were impregnated with varying concentrations of ethanolic fruit extract ranging from 1mg to 4mg per disc dissolved in DMSO and dried The discs were placed on yeast seeded plates and incubated at 37˚Cfor 48hrs.Disc impregnated with only 100% DMSO served as negative control Amphotericin B (100 units/disc) was used as positive control . Following an incubation period of 48hrs, plates were removed from the incubator and antifungal activity was evaluated by measuring zones of inhibition of fungal growth. Clear zone within which fungal growth was absent were measured and recorded as the diameter (mm) of complete inhibition. Experiments were conducted in triplicates

MIC of ethanolic fruit extract of *Terminalia bellerica*

The broth dilution method was used to determine the minimum inhibitory concentration of the extract against ***C neoformans*** Minimum inhibitory concentrations of the extract was prepared by serial doubling dilution of the extract to obtain concentrations in the range 3.3-1690µg/ml. Equal volume of the extract and Mueller – Hinton broth i.e. 0.5ml each were dispensed into sterilized test tubes. 0.1 ml of standardized inocula (3.3 X 10 CFU/ml) was added to each of the test tubes above. The tubes were incubated aerobically at 350C for 24 hrs. Tubes containing broth and plant extracts without inoculum served as positive control while tubes containing broth and inoculum served as negative control. The tubes were observed after 24 hrs of incubation to determine minimum inhibitory concentration, the lowest concentration that showed no evidence of growth (NCCLS, 2008)

TLC profile of ethanolic fruit extract of Terminalia bellerica:

TLC of crude extract was carried out on TLC glass plates coated with 0.2mm thickness silica gel 60 .10µl of crude extract was applied on the glass plates at equal distance with the help of micropipette. The plates were kept in a chromatographic chamber after drying. Chloroform: Ethyl acetate: Formic acid (2.0: 2.0: 0.8) was used as the solvent system. The plate was removed from the chamber, when the solvent front had reached the predetermined height and

the solvent front was marked precisely with pencil. Then the plate was dried and observed under UV light. The plate was sprayed with 5% ferric chloride in methanol. Rf of the band separated was noted

Phytochemical analysis of ethanolic fruit extract of *Terminalia bellerica*

Phytochemical screening of ***Terminalia bellerica*** for the presence of alkaloids flavanoids, hydrolysable tannins, coumarins, phenols, sugar etc ***was*** carried out as per standard procedures [4]

GC MS analysis of ethanolic fruit extract of *Terminalia bellerica*

The ethanolic extract was subjected to GC MS analysis [5] using the instrument GC MS Shimadzu QP2010 with GC MS solution version 2.53 software and Elite –DB-5M column Initially oven temperature was maintained at 70˚c for 2 minutes and the temperature was gradually increased up to 300˚C at 10/35 minutes and 4µl of sample was injected for analysis. Helium gas 99.995% of purity was used as a carrier gas as well as eluent. The flow rate of helium gas was set to 1.5ml/minute ,sample injector temperature was maintained at 260˚C and the split ratio is 20 throughout the experiment period .The ionization mass spectroscopic analysis was done with 70eV.The mass spectra was recorded for the mass range 40-1000m/Z for about 30minutes. The m/z ratio obtained was calibrated from the graph obtained which was called as the mass spectrum graph which is the fingerprint of the molecule. The identification of compounds was based on the comparisons of their mass spectra with NIST library 2008, WILEY8 & FAME

FTIR analysis was done to identify chemical groups present in the bioactive compounds The samples were scanned using infrared in the range 5000-500 cm-1 The spectral data obtained were compared with the reference chart to identify the functional groups present in the sample

RESULTS:

The dried fruit pulp of ***Terminalia bellerica*** was extracted with ethanol and the residue was recovered using a rotary evaporator

Anticryptococcal activity of ethanolic fruit extract of *Terminalia bellerica:* (Table 1 & 2)

The ethanolic fruit extract of Terminalia bellerica was tested for its antifungal activity against 5 clinical and 5 environmental isolates of C neoformans..At the highest concentration tested 4mg/ml, of all the 5 clinical isolates maximum zone of inhibition was shown by C3(18.5mm±0.65)followed by C4 (17.5mm±0.6) C1 & C2(16 mm ±0.5) and C5(15mm±0.5) Of all the 5 environmental isolates E3 showed maximum zone of inhibition (18±0.6) followed by E5 (17±0.65) and E2(13±0.6) E1&E4 showed less zone of inhibition comparatively (12±0.5).

MIC of ethanolic fruit extract of *Terminalia bellerica* :

The minimum inhibitory concentration of the extract against Cryptococcus isolates was determined by broth dilution method.The MIC for the clinical isolates C3 was found to be less **26.4µg/ml**. compared to other clinical isolates C1, C2 C4and C5 (52.8µg/ml) Among the environmental isolates the minimum inhibitory concentration of the extract was found to be **52.8µg/ml for E3,105.6 µg/ml for E2and E5,** whereas it was **211.25µg/ml** for **E1a**nd **E4**.The results were presented in Table3&4

TLC profile of ethanolic fruit extract of Terminalia bellerica:

TLC of the ethanolic extract was performed to separate active constituents in the extract . gallic acid was used as the standard. The Rf value was found to be 0.39.

Phytochemical analysis of ethanolic fruit extract of *Terminalia bellerica*

The phytochemical active compounds of Terminalia bellerica were qualitatively analysed and the results were presented in table. The ethanol extracts of Terminalia bellerica fruits showed the presence of phytochemical active compounds such as tannins quinines phenols coumarines and flavanoids and phytosterols etc (Table 5)

GC MS FTIR analysis of ethanolic fruit extract of *Terminalia bellerica*

GC MS analysis was carried out on the ethanolic extract of ***T.bellerica*** and 22 different compounds were identified. The chromatogram showed 22 peaks in the retention time range 4.06min to 19.44 min. The largest peak at 11.234 min with 57.45% area was identified as 1, 2, 3, benzenetriol The second less prominent peak at 17.000 min with 5.87%area corresponds to the compound Benzoic acid. The third prominent peak at 9.027 min with 4.39% area was due to the presence of. 5-Hydroxymethylfurfural followed by1 4H-Pyran-4-one, 2,3-dihydro-3,5-. (4.17%area) at retention time 7.720 min Other compounds identified were presented in Table 6 The IR spectral qualities of the fraction indicated the presence of ketones, aldehydes, carboxylic acids, amides, aromatic compounds alkanes alkenes alkynes and esters in their active components FTIR results were presented in Table 7

DISCUSSION

Mycotic infections are very difficult to eradicate and they constitute an enormous challenge for human health. The fungi, like their hosts, are eukaryotic organisms, making it difficult to choose intracellular targets whose inhibition would not also be deleterious to the host cells. Many of the current drugs are very toxic (Amphotericin B), lead to the development of resistance (fluconazole and 5-flucytosine) or exhibit drug-drug interactions (azoles)[6].Some Medicinal plants have therapeutic potential

due to the presence of natural antioxidants functioning as reducing agents, free radical scavengers and quenchers of singlet oxygen. Majority of their antioxidant activity is due to bioactive compounds viz. phenolic and polyphenolic compound

The present investigation was undertaken to evaluate the anticryptococcal property of T. bellerica fruits. Ethanolic fruit extracts were prepared and its anti cryptococcal activity was checked by disc diffusion method against clinical and environmental isolates of C .neoformans. Of all the clinical isolates C3 exhibited maximum zone of inhibition(18.5mm±0.65)and E3 was most susceptible(18mm±0.6) among environmental isolates at a concentration of 4mg.It was also found that clinical isolates were more susceptible than environmental isolates. Phytochemical investigation reported the presence of tannins, quinines, phenols,flavanoids, coumarines and phytosterols etc The bioactive components were identified by GC- MS .analysis . Identification of components was based on comparison of their mass spectra The identification of compounds was based on the comparisons of their mass spectra with NIST library 2008, WILEY8 & FAME

GC MS chromatogram showed 22 different compounds which contributed to the medicinal activity of the plant, with 1,2,3 benzenetriol showing maximum peak area 57.45%at a retention time 11.234 minutes. Benzenetriol is a pyrogallol with antiseptic , antioxidant, anti-dermatic, fungicide and candidicide[7] The antifungal property of pyrogallol was also reported by [8] examined the mechanism of the effects of green tea catechin which is a pyrogallol on *T. mentagrophytes* using electron microscopy and suggested that catechin attacked the cell membrane and caused lysis of the conidia and hyphae .the antifungal property of pyrogallol against ***Candida albicans***. has also been reported.[9] Benzenetriol was found to be an effective anti fungal, anti-microbial, anti-inflammatory and anti cancer agent [10]

Other compounds with prominent peak area are benzoic acid, followed by1 4H-Pyran-4-one, 2,3-dihydro-3,5-....these two compounds were found to possess antimicrobial and anti-inflammatory property .1 4H-Pyran-4-one, 2,3 dihydro-3,5 is a flavanoid with anti inflammatory, antimicrobial and anticancerous property Similar reports were given by[11]

Terminalia bellerica, is an important medicinal plant with diverse pharmacological spectrum.. The plant has many important phytoconstituents like Gallo-tannic acid , ellagic acid, gallic acid , thanni lignan, flavone and anolignan B, sitosterol, mannitol, glucose, fructose and rhamnose. These compounds were found to be responsible for many of the pharmacological activities such as antimicrobial, antioxidant, antidiarrhoeal, antidiabetic, analgesic,

immumomodulatory, and bronchodilatory activities[12][13]

Terminalia bellerica extract has immunosuppressant effects at low concentrations while stimulatory activity is observed at high concentrations. This suggest a potential therapeutic application of this plant in the treatment of disease associated with the functions of phagocytes and lymphocytes [14] since Cryptococcosis is an associated disease in immunocompromised patients like AIDS the drug is effective in modulating the immune system

Hence, this plant provides a significant role in the prevention and treatment of a disease. Further evaluation needs to be carried out to explore the concealed areas and their practical clinical applications, which can be used for the welfare of the mankind.

CONCLUSION

In the present study the ethanolic fruit extract of ***Terminalia bellerica*** showed significant anticryptococcal activity against clinical isolates. GC MS analysis revealed that bioactive components in the fruits of ***Terminalia bellerica*** were tannins and polyphenolic compounds. Benzenetriol and Pyran-4-one, 2,3-dihydro-3,5-....with typical antifungal activity were identified as predominant compounds Further analysis and fractionation of these pure compounds is needed to recommend the compounds for therapy

Table 1 Anticryptococcal activity of crude ethanolic fruit extract of *Terminalia bellerica* against clinical isolates

S No	ISOLATE	Zone of inhibition in mm				Amphotericin
		1mg	2mg	3mg	4mg	100units/disc
1	C1	4±1	12±0.5	14±0.65	16±0.5	12±0.5
2	C2	5±1	12±0.5	14±0.65	16±0.5	12±0.5
3	C3	7±0.5	13±0.5	15.5±0.5	18.5±0.65	13±0.5
4	C4	-	12±0.5	14±0.65	17.5±0.6	12±0.5
5	C5	-	10±0.5	13±0.5	15±0.5	12±0.5

Note: : Mean values of triplicates (zone of inhibition) ± S.D

Table 2 Anticryptococcal activity of crude ethanolic fruit extract of *Terminalia bellerica* against environmental isolates

S No	ISOLATE	Zone of inhibition in mm				Amphotericin
		1mg	2mg	3mg	4mg	100units/disc
1	E1	-	10±0.6	11±0.65	12±0.5	13±0.5
2	E2	-	10±0.5	12±0.5	13±0.6	12±0.5
3	E3	10.8±0.76	14±0.65	15±0.5	18±0.6	13±0.5
4	E4	-	6±0.5	7±0.5	12±0.5	12±0.5
5	E5	-	13±0.6	14±0.65	17±0.65	12±0.5

Note: : Mean values of triplicates (zone of inhibition)± S . D

Table 3 Minimum inhibitory concentration of crude ethanolic fruit extract of *Terminalia bellerica* for clinical isolates

Sr. No	ISOLATE	CONCENTRATION IN µg									
		3.3	6.6	13.2	26.4	52.8	105.6	211.25	422.5	845	1690
1	C1	+	+	+	+	--	--	--	--	--	--
2	C2	+	+	+	+	--	--	--	--	--	--
3	C3	+	+	+	-	--	--	--	--	--	--
4	C4	+	+	+	+	--	--	--	--	--	--
5	C5	+	+	+	+	--	--	--	--	--	--

+ indicates growth, -- indicates no growth

Table 4 Minimum inhibitory concentration of crude ethanolic fruit extract of *Terminalia bellerica* for environmental isolates

SR. No	ISOLATE	CONCENTRATION IN µg									
		3.3	6.6	13.2	26.4	52.8	105.6	211.25	422.5	845	1690
1	E1	+	+	+	+	+	+	--	--	--	--
2	E2	+	+	+	+	+	--	--	--	--	--
3	E3	+	+	+	+	--	--	--	--	--	--
4	E4	+	+	+	+	+	+	--	--	--	--
5	E5	+	+	+	+	+	--	--	--	--	--

+ indicates growth, -- indicates no growth

Table 5 Phytochemical analysis of Terminali bellerica

S.No	Phytochemical test	Inference
1	Carbohydrate	+
2	Tannins test	+
3	Saponin test -	-
4	Flavonoid test	+
5	Alkaloid test	-
6	Quinones	++
7	Glycosides test	-
8	Cardiac glycosides test	-
9	Terpenoids test	-
10	Triterpenoid	-
11	Phenols	++

12	Coumarins	++
13	Proteins	-
14	Steroids and Phytosterol	+
15	Phlobatannin	-
16	Anthraquinones	-

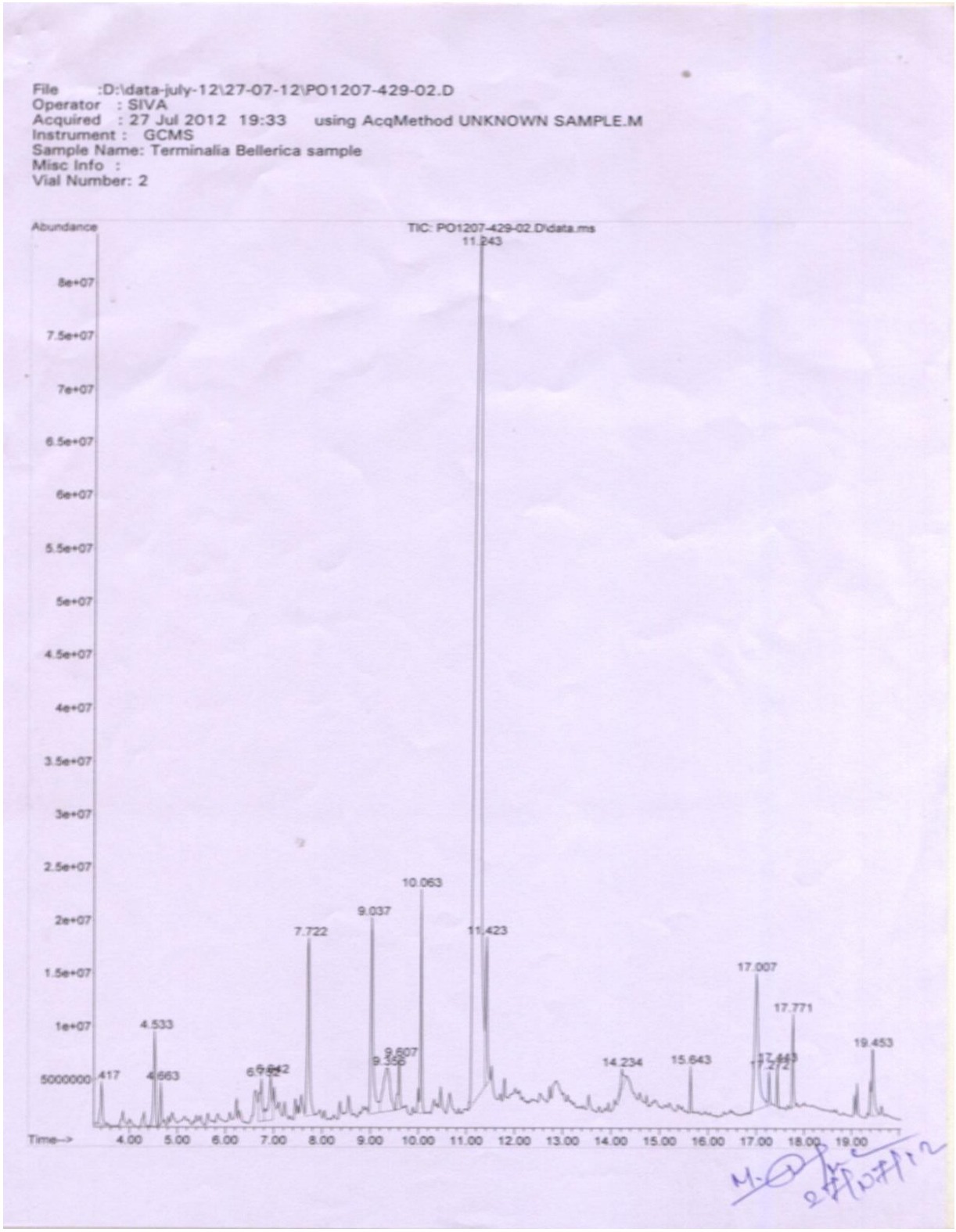
Table 6 Identification of compounds present in ethanolic leaf extract of Terminali bellerica by GC – MS analysis

S no	Peak No	Ret time	Area	Area %	Name of the compound
1	1	4.06	11413277	0.82	1 4-Cyclopentene-1,3-dione 2 3-Amino-1,2,4-triazine 3 Pyrimidine, 4-hydroxy-
2	2	4.525	25224372	1.81	1 2-Furanethanol, .beta.-methoxy-(S)- 2 p-Fluoroaniline 3 2(1H)-Pyridinone, 3-hydroxy-
3	3	4.655	9903909	0.71	2-Furancarboxaldehyde, 5-methyl
4	4	6.616	18050695	1.30	1 2,5-Furandicarboxaldehyde 2 Orcinol 10404 000504-15-4 50
5	5	6.747	11468960	0.82	6-Methyl-2-pyrazinylmethanol 5-Benzocyclooctenol, 5,6,7,8-tet... Pyrazine, 2-methoxy-6-methyl-
6	6	6.935	11040563	0.79	Undecane Undecane Decane, 2-methyl-
7	7	7.720	58041549	4.17	1 4H-Pyran-4-one, 2,3-dihydro-3,5-...
8	8	9.027	61165666	4.39	5-Hydroxymethylfurfural 2-Fluorobenzyl alcohol
9	9	9.346	39600145	2.84	1 5-Hydroxymethylfurfural 3 4-Mercaptophenol
10	10	9.608	14046593		1 4-Chlorobenzoic acid, undec-2-en... 2 Isonicotinic acid N-oxide

					3 N-Methyl-3,5-dihydroxyaniline
11	11	10.058	41472481	2.98	1 2,4-Difluorobenzoic acid, 5-dode... 2 2,4-Difluorobenzoic acid, 3-dode... 3 6-Methyl-2-mercaptopyridine-1-oxide
12	12	10.653	9269564	0.67	2-Methyl-3,4,5,6-tetrahydropyrazine 3-Methyl-2,5-oxazolidine-dione 2H-Imidazol-2-one, 1,3-dihydro-4...
13	13	11.234	799890819	57.45	1,2,3-**Benzenetriol**
14	14	11.423	47490398	3.41	Imidazole, 2-acetoxy- L-Proline, 5-oxo-, methyl ester DL-Proline, 5-oxo-, methyl ester
15	15	11.525	10138455	0.73	1,2,3-**Benzenetriol**
16	16	12.875	14812811	1.06	1 .beta.-D-Glucopyranose, 1,6-anhy... Heptanoic acid
17	17	14.226	56674439	4.07	Asarone 3 Benzene, 1,2,4-trimethoxy-5-(1-p...
18	18	14.589	9250737	0.66	Imidazole, 5-carbonylvinyl-4-nitro- 2 4a(2H)-Naphthalenecarboxylic aci...
19	19	17.000	81722945	5.87	Benzoic acid, 3,4,5-trihydroxy-,... 3,4,5-Trihydroxybenzhydrazide 3 Benzoic acid, 4-fluoro-3-methoxy...
20	20	17.261	10311717	0.74	Clorophene
21	21	17.770	20321063	1.46	1 n-Hexadecanoic acid
22	22	19.440	31031433	2.23	9-Octadecenoic acid, (E)- Octadec-9-enoic acid

Table 7 IR spectral qualities of fractions of ethanolic extract of *Terminalia bellerica*

S.No	Frequency Cm^{-1}	Nature of bond	Functional Group
1	3074.59	C-H aromatic ring	Alkenes
2	2921.24	C-H stretch	Alkanes
3	1743.68	C =O stretch	Aldehydes,ketones,carboxylic acids and esters
4	1644.34	-C=C - stretch	alkenes
5	1539.22	C=C	Aromatic ring
6	1453.39	C-H stretch	Alkanes
7	1369.48	C-N stretch	Aromatic amines
8	1246.04	C -O stretch	Aromatic ethers aryl-O-stretch
9	1041.58	C -O stretch	Aldehydes,ketones,carboxylic acids and esters
10	768.65	C-H stretch	Alkenes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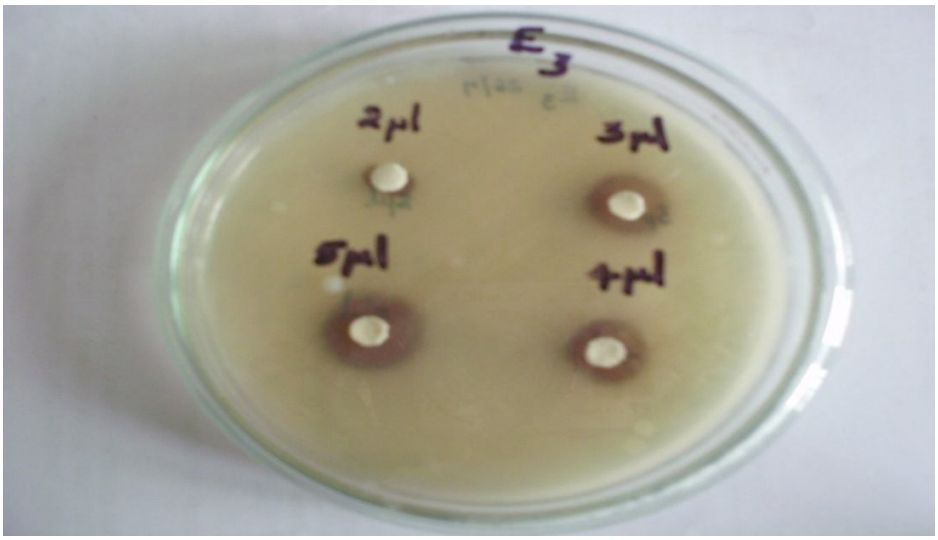

Fig 1 Disc diffusion assay of ***Terminalia bellerica*** ethanolic extract against environmental isolates of ***Cryptococcus neoformans***

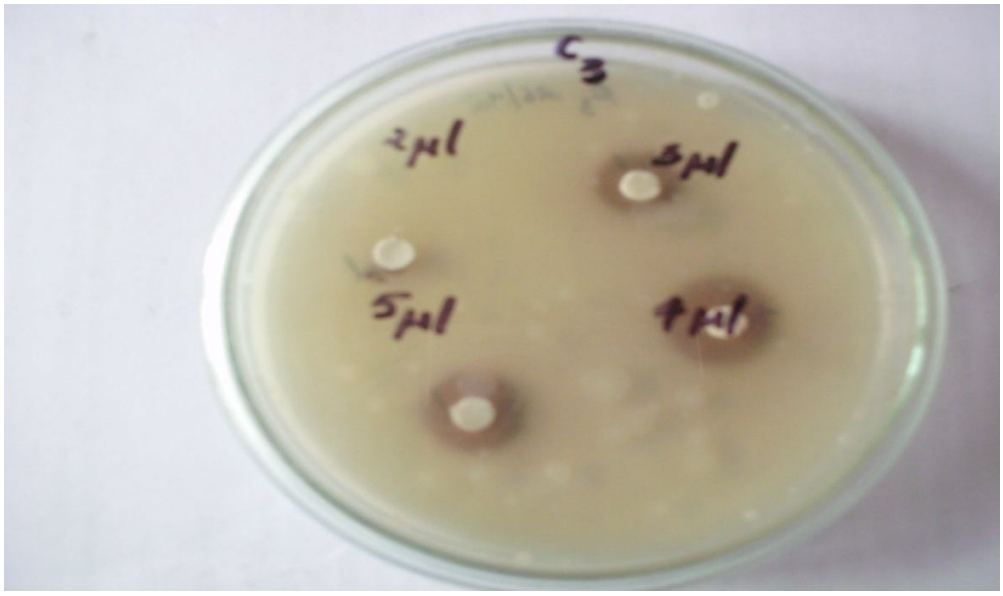

Fig 2 Disc diffusion assay of ***Terminalia bellerica*** ethanolic extract against clinical isolates of ***Cryptococcus neoformans***

REFERENCES :

1. Payne, J. W., Barrett-Bee, K. J. & Shallow, D. A. Peptide substrates rapidly modulate expression of dipeptide and oligopeptide permeases in *Candida albicans*. *FEMS Microbiol Lett* **1991; 79**: 15–20

2. Mallik j, Priyanka das Bijoy karon , Sourav das A review on phytochemistry and pharmacological activity of ***Terminalia bellerica*** International journal of drug formulation and research,2012 *3 (6),1-7*

3. Bauer AW , Kirby WMM, Sherris JC ., Turck, M Antibiotic Susceptibility testing by a standardized single diskmethod. *American journal of clinical Pathology*. 1966 **45**:493-496

4. Yadav RNS, Munin Agarwala. Phytochemical analysis of some medicinal plants. Journal of Phytology. 2011; 3(12): 10-14

5. Stanley A C, V Alex Ramani,A Ramachandran. Phytochemical screening &GC MS studies on the ethanolic extract of ***Cayratia pedata*** . *Int. J. Pharm. Phytopharmacol. Res* 2011.**1(3):**112-116

6. White MH, Bowden RA, Sandler ES, Graham ML, Noskin GA, Wingard JR, Goldman M van Burik J-A, McCabe A, Lin J-S, Gurwith M, Miller CB. Randomized,double-blind clinical trial of

7. Amphotericin B colloidal dispersion vs. Amphotericin B in the empirical treatment of fever and neutropenia. Clinical Infectious Diseases, 1998 27, 296-302.

8. Gopalakrishnan and vadivel. Gc-Ms Analysis Of Some Bioactive Constituents Of Mussaenda Frondosa Linn 2011, 313-320.

9. Toyoshima, Y., Okubo, S., Toda, M. . Effect of catechin on the ultrastructure of Trichophyton mentagrophytes. Kansenshogaku Zasshi1993 **68**, 295–303.

10. Hirasawa M , Takada.K. Multiple effects of green tea catechin on the antifungal activity of antimycotics against *Candida albicans Journal of Antimicrobial Chemotherapy* 2004 **53**, 225–229

11. John S A, Kumar. P GC-MS Study of the *Excoecaria agollocha* Leaf extract from Pitchavaram Tamilnadu, India. *Researcher* 2012; 4(6): 10-14.

12. 11Praveen Kumar P, Kumaravel S and C. Lalitha Screening of antioxidant activity, total phenolics and GC-MS study of *Vitex negundo* Afr J of Biochem Res Vol. 2010 4(7), 191-195

13. The Ayurvedic Pharmacopoiea of India,1st edition, Published by The controller of Publications, Civil Lines, New Delhi .2001; part-1,01: 252.

14. Amrithpal Singh Saroya. Herbalism phytochemistry and Ethan pharmacology, Science Publishers. 2011; 357-361.

15. 14 Aurasorn Saraphanchotiwitthaya, Pattana Sripalakit and Kornkanok Ingkaninan. Effects of *Terminalia belerica* Roxb. Methanolic extract on mouse immune response in vitro, Major International Journal of Science and Technology. 2008;02(2):400-407.