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Studies of Qualitative Phytochemical Screening and Effects of Different Solvent Extracted Samples of *N.arbortristis* Linn Leaves on the Growth of *C.albicans*.

Seema Kumari[1], Vinod Prasad[1] and Rashmi Komal[2]
[1]Department of Botany, B. N College, Patna University, Patna-800004, Bihar, India.
[2]Department of Botany, Patna Science College, Patna University, Patna-800004, Bihar, India.
Email ID- Seemakumaribt@gmail.com

ABSTRACT
Present study is based upon qualitative estimation and antimicrobial effects of three different organic as well as inorganic solvent extracted samples of N. arbortristis L. leaves against *the growth of C. albicans. Three different solvents methanol, ethyl acetate and hot water are used for preparation of plant extracts. Analysis of qualitative phytochemical screening indicate that all three different solvent extracted samples show presence of primary metabolites like carbohydrate, protein and lipids besides the primary metabolites some secondary metabolites, like alkaloids, phenol, terpenoids etc. were also found in different proportion. In present investigation agar well diffusion method was adopted to study the effects of extracted samples on the growth of C. albicans. 50% concentration of methanolic extracted samples of N. arbortristis leaf creates the zone of inhibition of 21mm in diameter and that in 100% concentration it creates negligible size of zone of inhibition. It means the extract of N. arbortristis leaves in 50% concentration is more effective than that of in 100% concentration. Similarly, ethyl acetate extracted samples in 50% as well as 100% concentration have equal effect on the growth of C. albicans and it creates a zone of inhibition of 13mm. Again hot water extracted samples in 50% concentration creates a zone of inhibition of 20mm whereas in its 100% concentration there was no zone of inhibition. Altogether, the methanolic extract of the leaves in its 50% concentration is more effective and it has more inhibitory effect on the growth of C. albicans.*
KEYWORDS: Agar well diffusion method, *C. albicans*, Inhibition zone, *N. arbortristis,* Phytochemical screening

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INTRODUCTION

Nyctanthus arbortristis Linn. is a valuable indigenous medicinal plant that belongs to the family Oleaceae. It is also known as night jasmine and its vernacular name is Harsinghar. In Sanskrit it is known as Parijata [1]. It is the trend of Indian mythology that all medicinally important plants were made associated with religion and ethics. Harsinghar is mythologically regarded as 'wish granting trees' in Devaloka [2]. Plant and plant products impart the basis for the research and development of new plant based herbal medicine [3].Since beginning of civilization human uses plants or their component for curing different diseases, for this purpose plant based herbal medicine play a crucial roles in primary health care [4]. Literature survey shows that different part of the plant provides various sources of secondary metabolites like alkaloids, phytosterols, glycosides, saponins, tannins, flavonoids and phenolics which are used for the treatment of different types of diseases [5]. Recent studies in antibiotic reveal that excessive use of antibiotic produces alarming effect on human health and some microbes develop resistance against some antibiotic. Therefore, it is necessary to develop a plant based drug which has no or feeble side effects on the patients. Medicinal aspects of *N. arbortristis* plant is limitless and each plant part may be used for the treatment of different diseases. The main focus of this study is to evaluate the effects of the plant on the growth of *Candida albicans* because this pathogen causes severe disease known as Candidiasis that affect mouth or throat, vagina, internal organ like kidney, heart or brain. Generally it has been seen that intake antibiotics to cure Candidiasis lowers the strength of the immune system and it aggravate the disease and make it more intense. So antibiotics are not more effective in this particular disease. With comparison to males, females are more susceptible to this disease [6]).

MATERIAL AND METHODS

The plant samples used in the study was fresh and shade dried leaves of *N. arbortristis* that has been obtained from the Garden of medicinal plants of B.N. College, Ashok Raj Path, Patna University, Patna during the month November 2019 to February 2020.This plant has been taxonomically identified by different literature and was authenticated by different sources.

The fungal strain named *C. albicans* (MTCC No 227) obtained from Institute of Microbial Technology (IMTECH), Chandigarh and was used in this experiment. Strains were sub cultured on SDA media for every 15 days and it was maintained on SDA slants at 4^{o}C.

To study the intensity of affectivity of different solvent extracted phytocompounds with comparison to standard antibiotics fluconazole(Stock 1mg/ml) for Candidiasis samples of three petriplates were treated with solution of fluconazole in its concentration of 20µg per disc (7).

Fresh leaves of the plant were washed with sterilized water and were dried in shade. The leaves were used in preparation of extract in three different solvents like methanol, ethyl acetate and hot water with the help of Soxhlet apparatus (8). Qualitative estimation of different solvent extracted samples of plant was done and the protocols applied in the estimation process were adopted from the book (9) and (10).

A small amount of different solvent extracted samples were dissolved in distilled water and the extract was filtered and subjected to Molisch's reagent (Mixture of α- napthol and 95% alcohol). Presence of reddish colored ring at the interface showed the presence of carbohydrate. Presence of glycosides was confirmed by appearance of pink colour in the sample of test tube by addition of mixture of chloroform and ammonia solution in definite amount of each extract.

1 ml. of test sample was taken in a test tube and 3ml. of Biuret reagent (Mixture of 5% NaOH and 1% $CuSO_4$) was added. Appearance of purplish violet colour indicated the presence of protein. Different samples were placed separately in between two filter papers and appearance of oil stain on filter paper indicated the presence of fat. Again confirmatory test was performed by Sudan IV test.

Each extract were dissolved in dilute HCl and they were filtered and further treated with Mayer's reagent (to prepare the reagent 1.36gm of mercuric chloride was added in 60 ml distilled water and 5gm of potassium iodide was added in 20ml distilled water and the final volume was made 100ml) and appearance of creamy precipitate indicated the presence of alkaloids. Presence of alkaloids was also confirmed by addition of Wagner's reagent (a mixture of 1.27 gm of iodine and 2 gm of potassium iodide in 5ml of distilled water) in the extract. Appearance of reddish brown precipitate confirmed about the presence of alkaloids.

Each extract were treated with 3-4 drops of $FeCl_3$ solution and the appearance of reddish colour precipitate indicated the presence of phenols. Presence of terpenoids was confirmed by the development of a reddish brown colour at the interface after addition of 2ml of chloroform ($CHCl_3$) and 3ml of H_2SO_4.in the extract. Test samples were diluted with sterilized distilled water and they were kept for 15 minutes in a test tube. Appearance of about one centimeter thick layer of foam indicates the presence of saponins.

2ml of each extract were treated with few drops of acetic anhydride and conc. H_2SO_4 were added from the side wall of test tube. The solution were shaken and allowed stand for 15 minutes. Appearance of bluish green colour at the lower layer of test tube indicated the presence of steroids. 2ml of each extract were treated with lead acetate solution and after that 1% solution of gelatin containing 10% sodium chloride were added to the solution. Appearance of white coloured prepcipitate indicated the presence of tannin.

Again test samples were treated with few drops of lead acetate solution and appearance of yellow precipitate indicated about the presence of flavonoids.

Plant extract preparation: 29 gram of dry powered of *N. arbortristis* was loaded in the thimble of Soxhlet apparatus and 300ml of solvent was put into the appropriate size round bottle flask and the upper part was fitted with condenser. A Mantox heating mental was used for the constant supply of heat and recycling of solvent. After completion of extraction process the samples were transferred into aliquots.

Agar well diffusion technique was used for the study of antimicrobial assay (11). Sabouraud Dextrose Agar media was prepared and sterilized at 121^{0}C temperature for 15 lbs pressure in autoclave. *Candida albicans* culture was spread onto the SDA media and after that a well of 6mm diameter was created with the help of sterilized micropipette. Four different concentrations (25%, 50%, 75%, and 100%) of samples were prepared and introduce the sample onto the four different wells on the Petri plate and was incubated at 25^{0}C ± 2^{0}C temperature for 72 hours. Diameter of inhibition zone was measured by zonal scale in mm.

RESULT AND DISCUSSION

Qualitative phytochemical screening of the different solvent extracted samples of *N. arbortristis* were performed and all the solvent extracted samples were found to have presence of carbohydrate, protein and fats whereas secondary metabolites like alkaloids, phenolic, steroid, tannin and steroids were found to have varies in their proportion. Alkaloids and tannin were found to have absent in ethyl acetate and hot water extracted samples whereas they were present in methanolic extracted samples. Steroids were present in hot water extracted sample and were absent in methanolic and ethyl acetate extracted samples (table 1).

Agar well diffusion method was adopted for the study of effect of different solvent extracted samples of *N. arbortristis* leaves on the growth of *C. albicans*.The maximum zone of inhibition was seen in methanolic extracted samples at the 50% concentration (figure 3) whereas ethyl acetate and hot water extracted samples shown maximum zone of inhibition at concentration of 50% and it was found to have 13 and 20 mm respectively (figure 1and 2). Methanolic and hot water extracted samples showed minimum zone of inhibition at concentration of 100% (figure 3 and 2). On the other hand ethyl acetate extracted samples showed minimum zone of inhibition and it was found to have 11 mm at 25% concentration (figure 1).

Although the size of inhibition zone induced by fluconazole (34mm, figure 4) is no doubt greater than that of the induced by different solvent extracted herbal medicine or phyto-medicine but the side effects of phyto-medicine or herbal medicine is negligible whereas that of the fluconazole is more intensified and to some extent it work as immunodeppresent due to which the *C. albicans* appears as more aggravated form. That's why it may be recommended as a mode of phytotherapy than that of the fluconazole.

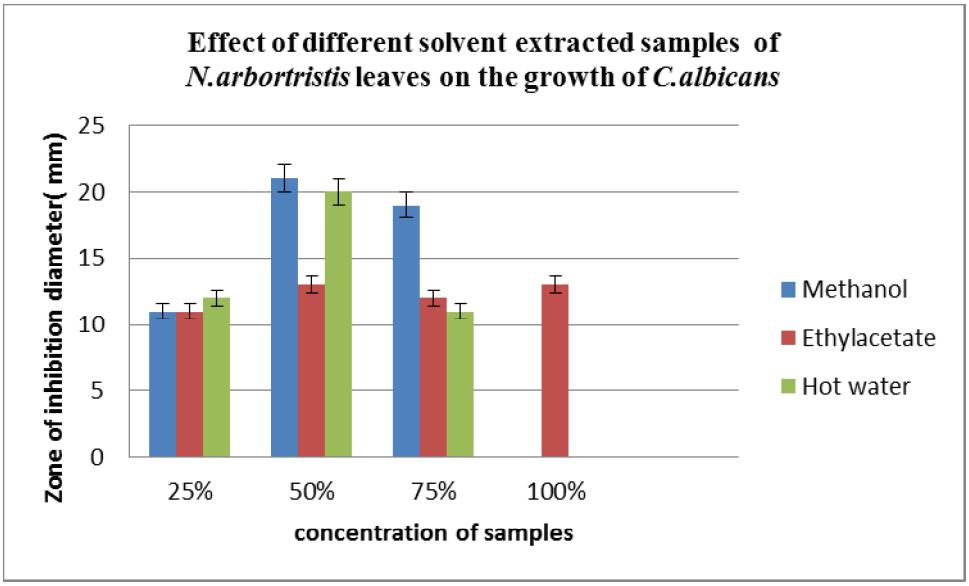
Most of the infectious diseases are treated by the use of antibiotics and tremendous use of this antibiotic produces many side effects like loss of micro flora inside gut, allergic reaction and it weakens the immune system of host and side by side other health problems may also arises. In present scenario there is a need of development of herbal medicine with no or feeble side effect and it should be economical also. Medicine based on plant origin is of great importance to treat different diseases. Present study concludes that different types of secondary metabolites which are the constituent of herbal or phytomedicine are present in *N. arbortistis* leaves. Different studies suggested that the different part of this plant has been used for different purposes like control of sugar level, immunostimulatory activity, analgesic and antipyretic effects. Thus based on present research this plant part can be used for the preventive measure of Candidiasis.

Table 1 Qualitative phytochemical screening of three different solvent extracted samples of *N. arbortristis*

Phyto- constituents	Methanol	Ethyl acetate	Hot water
carbohydrate	+	+	+
Protein / amino acid	+	+	+
Fat/lipid	+	+	+
Phenolic	+	+	-
Glycosides	+	+	+
Alkaloids	+	-	-
Terpenoids	+	+	+
Steroids	-	-	+
Saponins	+	+	+
Flavonoids	+	+	+
Tannins	+	-	-

Table 2 Zonal diameter of three different solvent extracted N. arbortristis leaves against C. albicans at four different concentrations

Plant extract	Dilution of plant extract(1mg/ml)												
	methanol				Ethyl acetate				Hot water				standard Antibiotics fluconazole (1mg/ml)
conc	25 %	50 %	75 %	100 %	25 %	50 %	75 %	100 %	25 %	50 %	75 %	100 %	
	Zone of inhibition(mm)												
N. arbortristis Linn.	11	21	19	00	11	13	12	13	12	20	11	00	34mm



Graph 1- Zone of inhibition of three different solvent extracted samples of *N. arbortristis* leaves against *C. albicans* growth at four different concentrations.

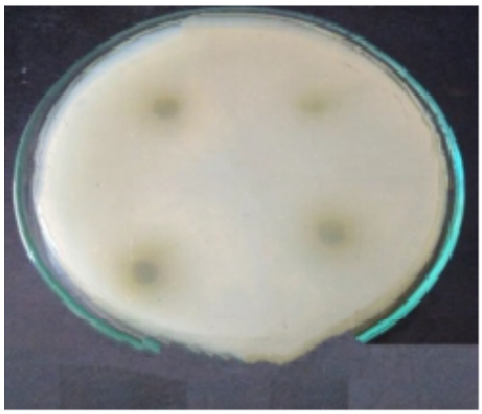


Figure 1 Diameter of zone of inhibition of ethyl acetate extracted *N. arbortristis* leaves against *C.albicans*

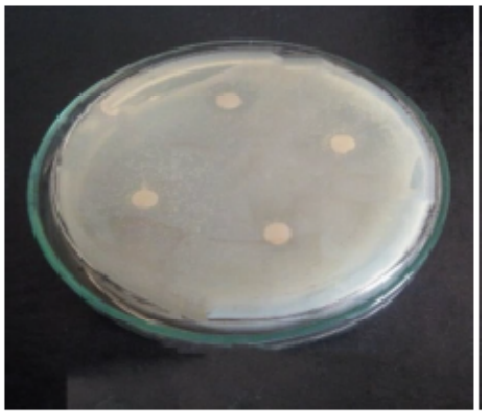


Figure 2 Diameter of zone of inhibition of hot water extracted *N. arbortristis* leaves against *C. albicans*

Figure 3 Diameter of zone of inhibition of methanolic extracted *N. arbortristis* leaves against *C. albicans*.

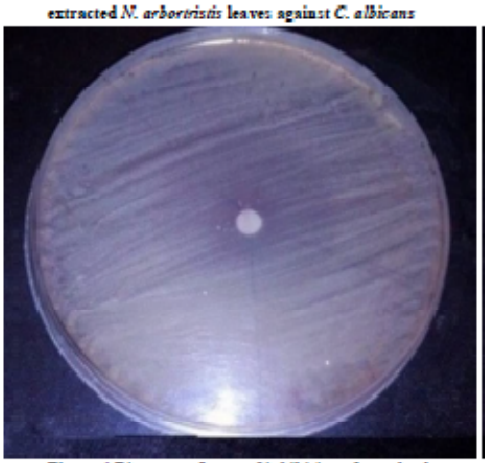


Figure 4 Diameter of zone of inhibition of standard antibibiotics fluconazolle against *C.albicans*

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CONFLICT OF INTEREST

I don't have any conflict of interest during my completion of research work.

CONCLUSION

Present work provides useful and innovative idea for the formulation of plant based drugs for Candidiasis. This study also suggests about use of plant products for prevention of fungal infections.

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