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ANTIMICROBIAL, ANTIFUNGAL AND ANTIDIABETIC PROPERTIES OF *CLERODENDRUM INFORTUNATUM*

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ABSTRACT

Clerodendrum infortunatum leaves extract have been studied for their potential antibacterial, antifungal, free radical scavenging, α amylase and α glucosidase activity. The properties of ethanol and chloroform extracts were analyzed by *in-vitro* techniques. The antimicrobial activity of *C.infortunatum* was performed by disc diffusion method in which the ethanol extract was exhibited significant antibacterial activity against *Bacillus subtillis* (12.00mm), *Aeromonas hydrophila* (4.67 mm), *Shigella boydii* (10.67 mm), *Escherichia coli* (8.00 mm) and *Staphylococcus.aureus* (10.00 mm), and antifungal activity against *Trichophyton mentagrophytes* (9.00mm), *Fusarium oxysporum* (13.00mm), *Penicillium chrysogenum* (11.34mm), *Aspergillus niger* (14.67mm) and *Aspergillus flavus* (7.34mm). The qualitative analysis of photochemical showed the presence phenolics, alkaloids, flavonoid, saponin, tannin and anthraquinone on both ethanolic and chloroform extracts. Furthermore, to evaluate α-amylase and α-glucosidase inhibitory effect of *C. infortunatum* extracts, the extracts were subjected to *in-vitro* inhibition studies with the enzymes α-amylase and α-glucosidase. The ethanolic extract of *C.infortunatum* showed high inhibition in both α-amylase (17.25%) and α-glucosidase activities. The results provided a platform for the use of *C. infortunatum* for therapeutics.

KEYWORDS: ***Clerodendrum infortunatum,*** **anti bacterial, antifungal, α-amylase.**

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INTRODUCTION

Many plant products play a vital role in the discovery of therapeutic medicines[1].These preventive properties have been attributed to the presence of flavonoids and other polyphenolic compounds which may exert their effects as a result of antioxidant activity [2].The genus *Clerodendrum* is widely distributed in Asia, Australia, Africa and America. The major chemical components reported from the genus are phenolics, steroids, triterpenoids, flavonoids, volatile oils, etc.,[3]. There are more plant species available which has the constituents for therapeutic purpose but it needs systematic investigations for the estimation of their pharmacological activities. Keeping this in view, coupled with the fact that the genus *Clerodendrum* has a high content of secondary metabolites including flavanoids and phenolic compounds.Antioxidants involves in the protection of cell damage by reactive oxygen species[4]. Cardiovascular and neurological disorders are influenced by reactive oxygen species (ROS) and other free radicals [5]. An Increase in such diseases can therefore be delayed by minimizing redox imbalances [6]. Antioxidants are scavengers of ROS and other free radicals by acting as substrates for oxidation which in turn terminates the chain reactions [7]. Natural antioxidants with more specificity would be more potent but less toxic than the commercial synthetic antioxidants [8].People with *diabetes mellitus* (DM) are increasing due to population, ageing, urbanization and lifestyle changes [9]. *Diabetes mellitus* has become a major public health issue in developed and in developing countries [10,11]. *Diabetes mellitus* complication can be cured by many insulin secretion agents, aldose reductase inhibitors, R-glycosidase inhibitors, and biguanides. But the management of DM is feasible by the development of indigenous medicinal plant resources as practical and cost-efficient alternatives. Keeping this in view, the present study was designed by selecting *Clerodendrum infortunatum* to estimate the antioxidant, antidiabetic and antimicrobial properties.

MATERIALS AND METHODS

2.1. Raw material collection

The leaves of *Clerodendrum infortunatum* were collected from Wardha, Hinghanghat District, Maharashtra. The obtained leaves were washed thrice with distilled water to remove contaminants, and then it was dewatered by sieving through 2-mm mesh cloth. The washed samples were air dried for approximately 48h under shade. Dried leaves were ground in a laboratory blender for obtaining fine powder. The powder was sieved using sieve (ASTM 80 mesh size) which contained 0.25-0.18mm size particles. The sieved powder was stored in sealed plastic bags at room temperature.

2.2. Extraction

About 50mg of the powder was introduced into a lab scale soxhlet extractor 250mL round bottom flask and a condenser. The extraction was performed for 8h using 150 mL of two different solvents ethanol and chloroform respectively. After the extraction, the extract was concentrated by using a rotary evaporator until complete removal of the solvent was attained. The extracted semisolid substance was weighed and the extract obtained was expressed in weight percentage.

2.3. Phytochemical analysis

The obtained extract of both solvents were used to prepare untreated plant extract control (1mg/mL of DMSO). About 1 mL of control sample was transferred to test tubes to analyze the presence of phytochemicals in extracts. For determination of tannins and saponins 0.1% ferric chloride solution and distilled water was used respectively. Flavanoids were determined using a pinch of $MgCl_2$ and a few drops of concentrated hydrochloric acid were added. Alkaloids, proteins, steroids and anthraquinones were determined by adding few drops of Dragandoff reagent, Bradford's reagent, 10% sulfuric acid and aqueous ammonia respectively. The change in color was compared with control solution, the respective

color changes confirms the presence of phytochemicals in plant extract.

2.4. Antioxidant activity

The DPPH free radical scavenging potential of the extract was determined by using the modified method of Ching H *et al*., (2011) [6]. The percentage of inhibition of DPPH radical was obtained by analyzing the absorbance at 517 nm and it was calculated according to the equation.

$$Inhibition(\%) = \frac{Blank\ absorbance - test\ absorbance}{Blank\ absorbance} \times 100 \quad \ldots\ldots\ldots\ldots (1)$$

Ferrous reducing antioxidant property (FRAP) was analyzed by using the modified method of Ching H *et al*., (2011) [6].

2.5. Antimicrobial activity

2.5.1. Antibacterial activity

Bacterial strains such as *Escherichia.coli, Staphyloccus aureus, Bacillius subtilis, Aeromonas hydrophila, Shigella boydii* were used for assessing the antibacterial activity. Antibacterial activity of leaf extract was analyzed by the well diffusion method against above mentioned organisms. Nutrient broth containing 24h old cultures of above mentioned organisms was used for the assay. Using a sterile cotton swab, the culture was swabbed over the surface of Muller Hinton Agar plates and allowed to dry over the surface. The four wells were punched on to the agar surface loaded with 20 µl of both extracts (100mg of extract/mL dissolved in 1 mL of DMSO) of along with antibiotic (chloroamphenicol (100 mg/mL)) were added to respective wells. DMSO was used as a control. The plates were incubated at 37°C for 24 h. After incubation, the zone of inhibition was measured and recorded.

2.5.2. Antifungal activity

Five fungal strains such as *Aspergillus flavus, Aspergillus niger,Penicillium chrysogenum, Trichophyton mentagrophytes, Fusarium oxysporum* were used for assessing the antifungal activity. Antifungal activity of leaf extract was also analyzed by the well diffusion method against above mentioned organisms. Potato Dextrose broth containing 48h old cultures of above mentioned organisms were used for the antifungal assay. Using a sterile cotton swab, the culture was swabbed over the surface of Potato Dextrose Agar plates and allowed to dry over the surface. The four wells were punched on the agar surface loaded with 20µl of ethanolic and chloroform extracts along with antibiotic (Amphotericin B, 100mg/mL) were added to respective wells. DMSO was used as a control. The plates were incubated at 37°C for 24 h. After incubation, the zone of inhibition was measured and recorded.

2.6. Antidiabetic analysis

2.6.1. In-vitro inhibition of α-glucosidase.

Five hundred microliters (different concentrations) of both the extract and 1 mL of tris buffer pH 7, containing 100 µl (100mg/mL) of glucose and 100 µl (100mg/mL) of yeast α-glucosidase were incubated at 37°C for 60 minutes. 500 µl of acarbose in distilled water was taken separately and incubated as a control. The reaction mixtures were stopped by heating 2 min in a boiling water bath. The amount of liberated glucose was measured by the glucose oxidation method. Absorbance (A) was measured at 540nm. Percent inhibition was calculated as follows:

$$Inhibition\ (\%) = \frac{Enzyme\ activity\ of\ extract - Enzyme\ activity\ of\ control}{Enzyme\ activity\ of\ control} \times 100 \quad \ldots\ldots\ldots(2)$$

Control incubations represent 100% enzyme activity. Data points were means of triplicate values with error bars showing standard deviations.

2.6.2. In-vitro inhibition of α-amylase

Ethanolic and chloroform extracts (100mg) was suspended in 1mL of DMSO from that 500 µl of both the extracts were added in different tubes and introduced in to a test tube containing 500 µl of starch solution and 500 µl porcine α-amylase (27.5mg/100mL) and 500 µl of acarbose solution (100mg/mL) as taken as control. All these reaction mixtures were then incubated 25°C for 10 min. The reaction was stopped by adding 500 µl of DNS (3,5 di-nitro salicylic acid solution(96mM)) solution to all the test tubes. The tubes was then incubated in a boiling water bath for 5 min and cooled at room temperature. Absorbance (A) was measured at 540 nm. Percent inhibition was calculated as follows:

$$\text{Reaction}\ (\%) = \frac{\text{test}\ (\text{Maltose})}{\text{control}\ (\text{Maltose})} \times 100 \quad \ldots\ldots\ldots\ldots (3)$$

$$\text{Inhibition}\ (\%) = 100 - \%\ \text{reaction} \quad \ldots\ldots\ldots\ldots\ldots (4)$$

RESULTS

3.1. Phytochemical evaluation of extracts

The ethanolic and chloroform extracts of *C. infortunatum* were analyzed for the presence of phytochemicals and the detailed results on phytochemical analysis are presented in Table 1. As shown in table, the presence of tannins, saponins, flavanoids, alkaloids, proteins, steroids and anthraquinones were observed.

S. no	Phytochemicals	Ethanolic extract	Chloroform Extract
1.	Tannins	Positive	Positive
2.	Saponins	Positive	Positive
3.	Flavonoids	Negative	Negative
4.	Alkaloids	Positive	Positive
5.	Proteins	Positive	Positive
6.	Steroids	Positive	Positive
7.	Anthraquinones	Negative	Negative

Table 1
List of phytochemical constitution

3.2. Antioxidant activity

The DPPH scavenging activity was analyzed for both ethanol and chloroform extracts. The ethanol extract showed more scavenging activity compared to chloroform extract. The present finding certainly indicates both the extracts have the proton donating ability and could serve as free radical inhibitors. This study also suggests that the phenolic compounds contributed significantly to the antioxidant activity of *C. infortunatum* and the results were given in Fig.1 (a).Estimation of FRAP assay was done from both ethanol and chloroform extracts and the extract was showing ferric reducing antioxidant power and was shown in Fig.1 (b). The ethanol extract showed more ferric reducing antioxidant power compared to chloroform extract.

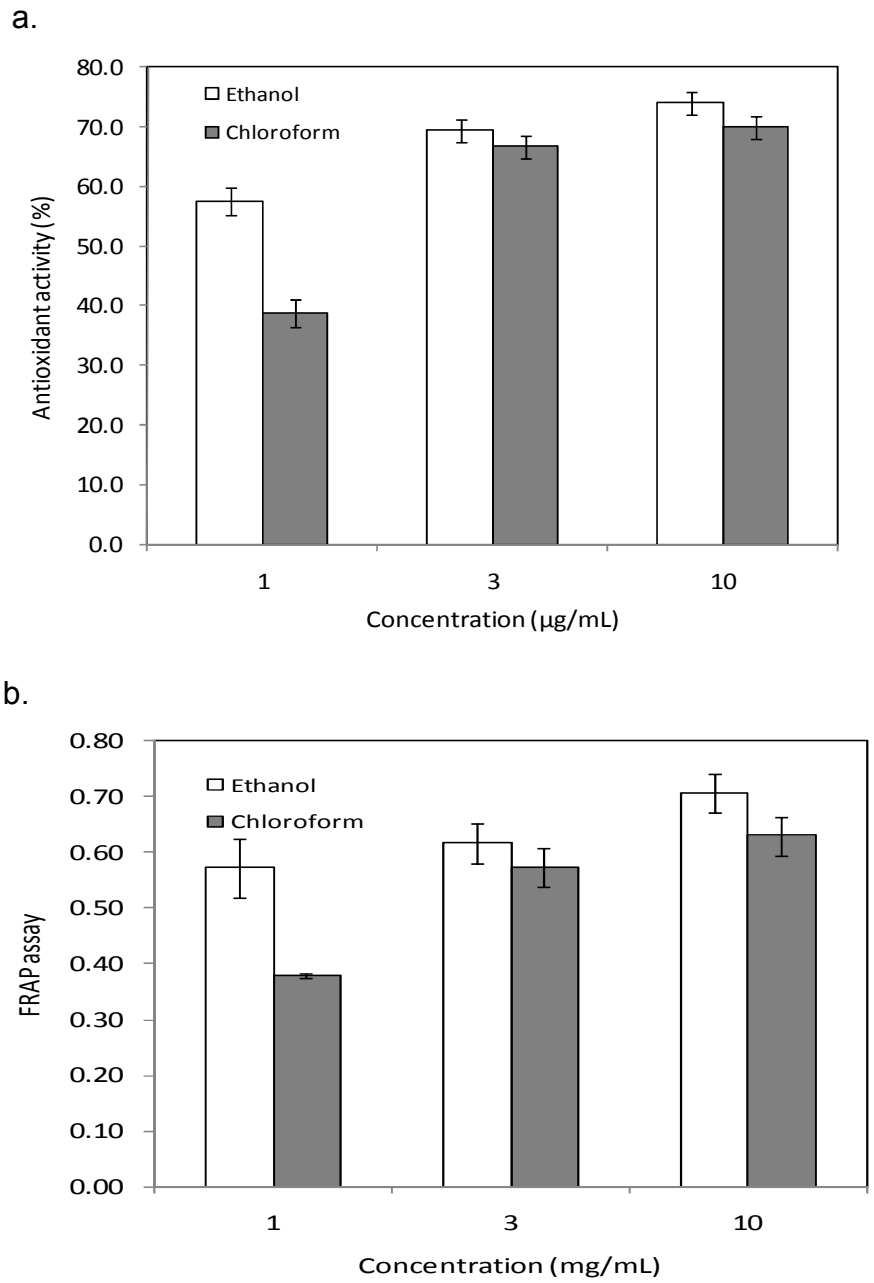


Figure 1
a. DPPH assay and b. FRAP assay of the ethanolic and chloroform extracts

3.3. Antimicrobial activity

The results of antibacterial and antifungal activity of both chloroform and ethanolic extracts (100 mg/mL) of *C. infortunatum* were given in Table 2 and Fig. 2, 3, 4 and 5 with respect to standard drug (100 mg/mL). From the results, it was found that both ethanolic and chloroform extracts exhibited significant antimicrobial activity. The ethanol extract exhibited significant antibacterial activity against *Bacillus subtillis* (12.00mm), *Aeromonas hydrophila* (4.67 mm), *Shigella boydii* (10.67mm), *Escherichia.coli* (8.00mm) and *Staphylococcus.aureus* (10.00mm), and antifungal activity against *Trichophyton mentagrophytes* (9.00mm), *Fusarium oxysporum* (13.00mm), *Penicillium chrysogenum* (11.34mm), *Aspergillus niger* (14.67mm) and *Aspergillus flavus* (7.34mm).

Microorganisms	Diameter of zone of inhibition (mm)		
Bacteria	Ethanolic extract	Chloroform extract	Antibiotic
Bacillus subtillis	12.00	9.34	17.34
Aeromonas hydrophila	4.67	7.67	18.00
Shigella boydii	10.67	8.00	15.67
Escherichia.coli	8.00	6.67	18.00
taphylococcus.aureus	10.00	9.34	33.00
Fungi	Ethanolic extract	Chloroform extract	Antibiotic
Trichophyton mentagrophytes	9.00	10.67	10.67
Fusarium oxysporum	13.00	8.34	11.67
Penicillium chrysogenum	11.34	10.34	10.00
Aspergillus niger	14.67	14.34	18.00
Aspergillus flavus	7.34	6.34	9.00

Table 2
Antimicrobial activity of Clerodendrum Infortunatum 8 9*- Leaves extract on bacteria and fungi.

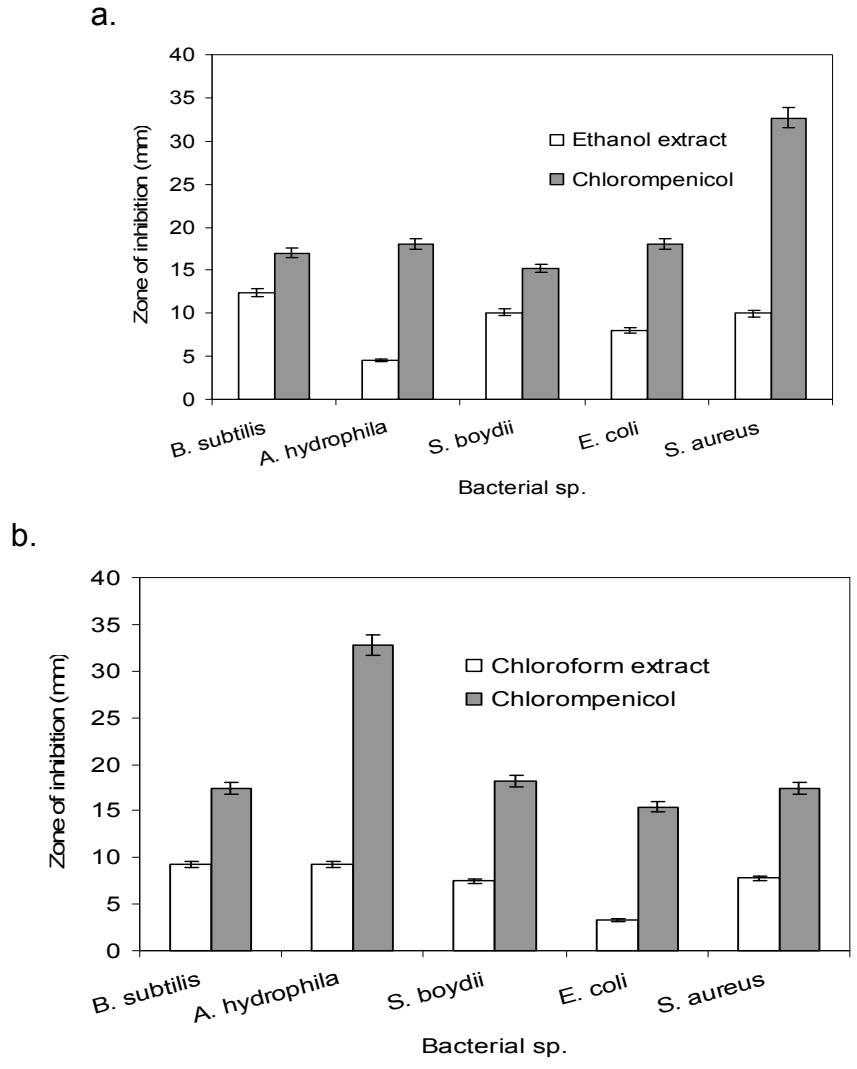


Figure 2
Results of Plant Extracts on bacterial strains for a. Ethnolic extract and b. chloroform extract

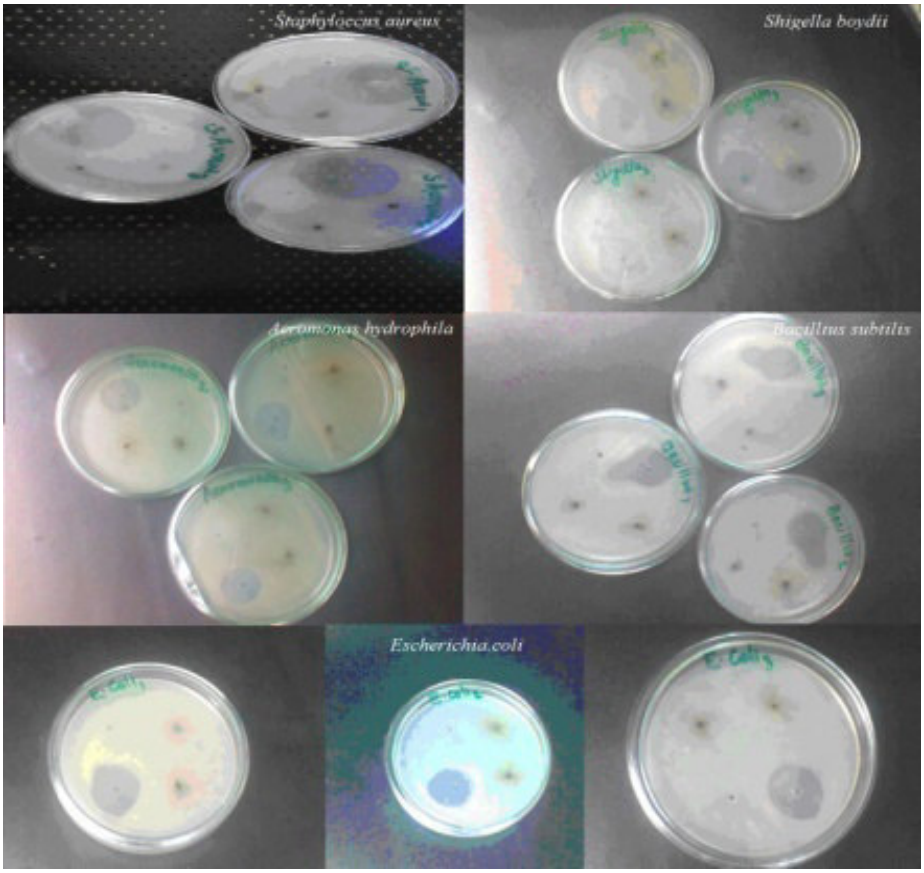
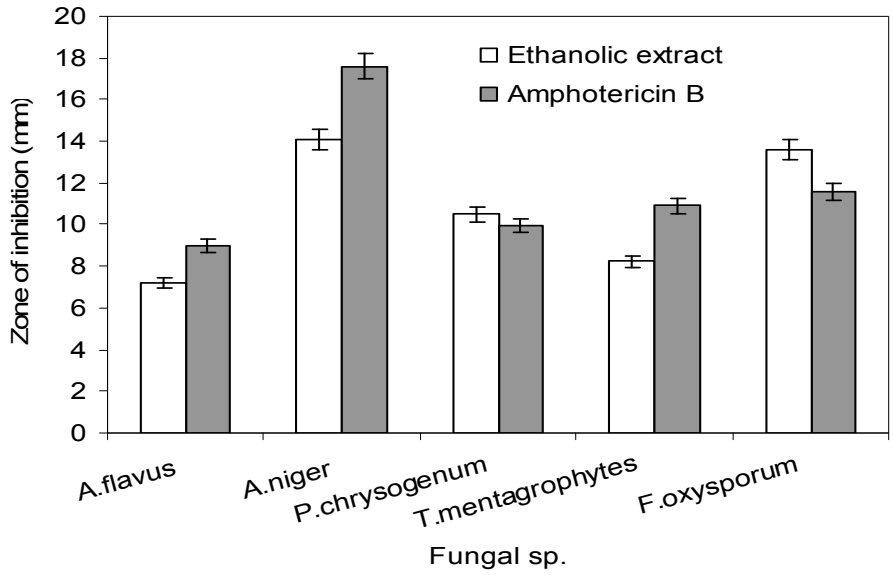


Figure 3
Antibacterial effect of plant extract on bacterial strains

a.



b.

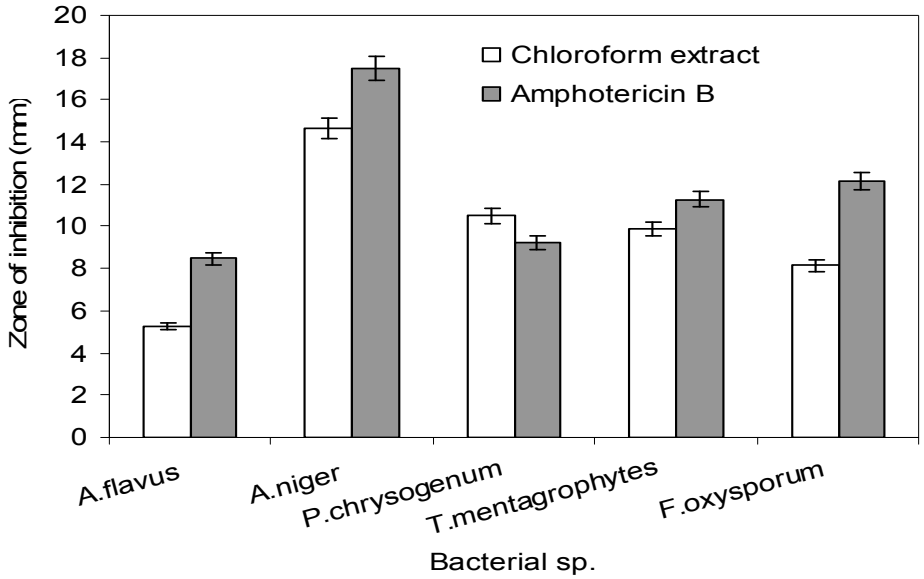


Figure 4
Results of plant extracts on fungal strains for a. Ethnolic extract b. chloroform extract

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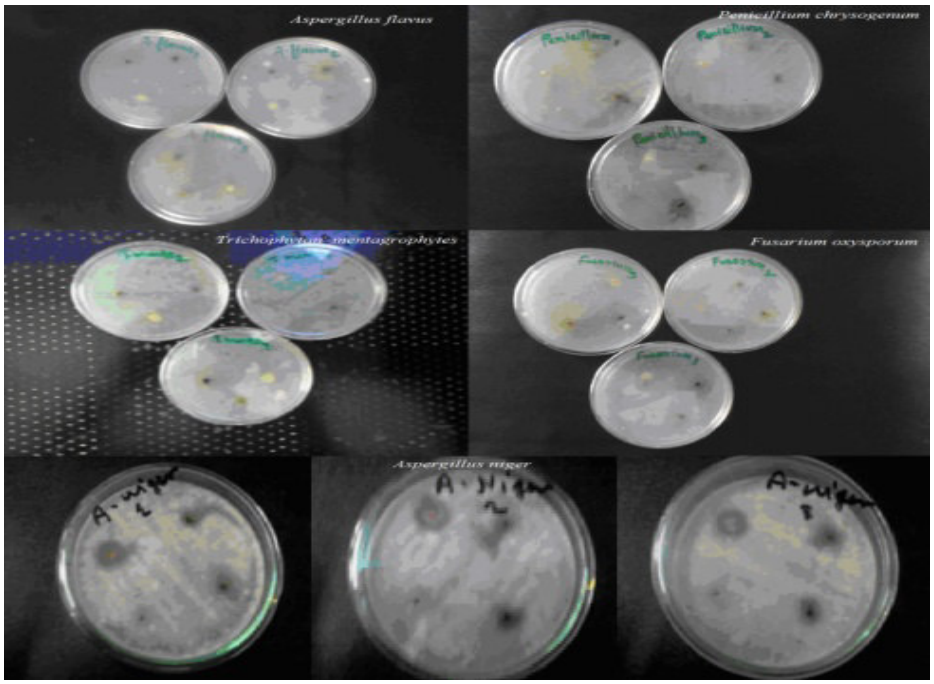


Figure 5
Antifungal effect of plant extract on fungal strains

3.4. Anti-diabetic activity

3.4.1. In-Vitro Inhibition of α-amylase

Both ethanolic and chloroform extracts of *C. infortunatum* produced a weak α-amylase inhibition. The maximum inhibition was in ethanolic extract 17%. The results obtained were given in Table 3 (a). It can be clearly seen that ethanolic extract demonstrated high inhibitory activity of α-amylase when compared to chloroform extract. This concludes that leaves of *C. infortunatum* possess α-amylase inhibitory effect.

3.4.2. In-Vitro inhibition of α-glucosidase

The *in vitro* α-glucosidase inhibitory studies demonstrated that *C.infortunatum* of both ethanolic and chloroform extracts had α-glucosidase activity. The percentage inhibition at 100, 50, 25, 10 mg/mL concentration of ethanolic extract showed a concentration-dependent reduction in percentage inhibition. Thus the highest concentration of 100mg/mL tested showed a maximum inhibition of nearly 31%. The percentage inhibition varied from 31-14% from the highest concentration to the lowest concentration of 10mg/mL. Chloroform extract showed a strong inhibitory potential with percentage inhibitions ranging from 29-14% for concentrations ranging from 100 – 10 mg/mL. Fig. 6 and Table 3 (b) illustrate the inhibitory activity of ethanolic and chloroform extract against α-glucosidase.

a.

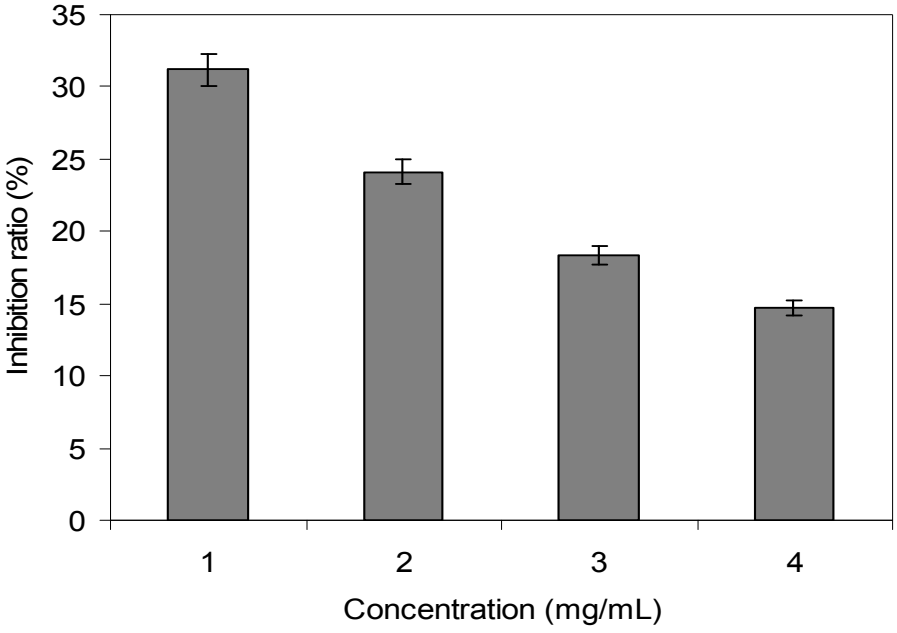
S.no.	Extract	Reaction (%)	Inhibition (%)
1.	Ethanolic extract	82.75	17.25±0.01
2.	Chloroform extract	89.65	10.35±0.02

b.

S. No.	Sample	Concentration (mg/ml)	Inhibition ratio (%)
1.	Ethanolic	100	31.14± 0.04
	Extract	50	24.07± 0.07
		25	18.51± 0.07
		10	14.81± 0.09
2.	Chloroform	100	29.62± 0.06
	Extract	50	25.92± 0.05
		25	20.37± 0.02
		10	14.81± 0.05

Table 3
Inhibition percentage of a. α-amylase and b. α-glucosidase with plant extract.

a.



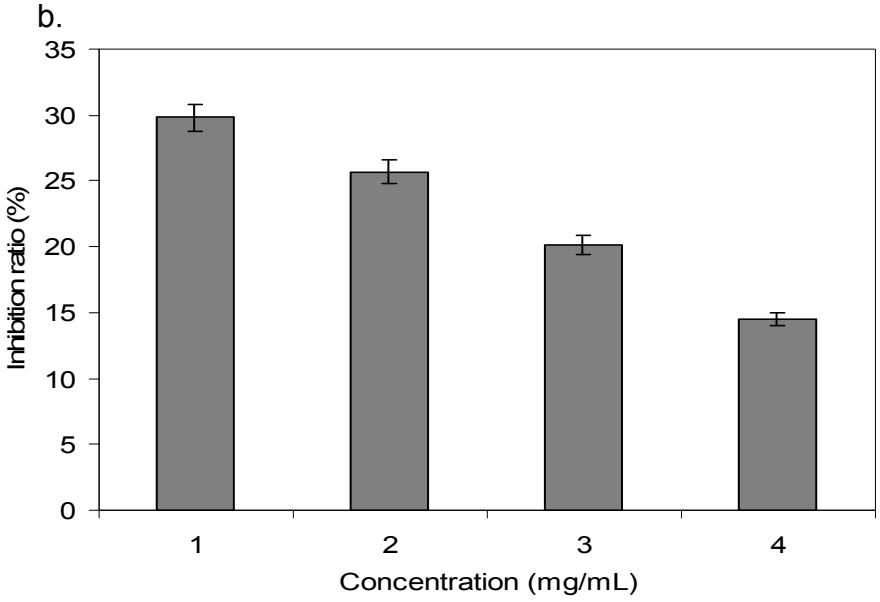


Figure 6
Results of in-vitro inhibition of α - glucosidase for a. Ethanolic extract b. Chloroform extract

DISCUSSION

Phytochemical analysis was performed on *C. infortunatum* leaves showed that tannins, saponins, alkaloids, steroids are major components of the extract. Compounds such as saponins are believed to be anti-feedants and protects the plant from microbes and fungi. From the results obtained, it can be clearly understood that both ethanolic and chloroform extracts exhibited significant antimicrobial activity. In case of bacterial strains, chloroform extract of *C. infortunatum* showed moderate results against *Shigella boydii*. When the zone of inhibition against bacterial and fungal species were compared, it was found that both extracts showed greater zone of inhibition against fungal species. This states that the extract has a greater inhibitory effect against fungal species. From these results, we can conclude that active components present in these extracts exhibit antimicrobial activity. From the results of phytochemical analysis, it was known that compounds such as alkaloids and steroids are major constituent of *C. infortunatum*. Sterols helps in reducing glucose level by down regulation of glucose 6-phosphate. Both ethanolic and chloroform extracts have shown inhibition of enzymes such as α-amylase and α-glucosidase. It was found that ethanolic extract demonstrated high inhibitory activity of α-amylase when compared to chloroform extract. In case of α-glucosidase inhibition, there was decrease in activity against α-glucosidase enzyme with a decrease in extract concentration. All the results were tested for significance and the value of significance was found to be > 0.05.Treating diabetes can be done by decreasing postprandial hyperglycemia. This can be done by retarding the absorption of glucose through inhibition of α-amylase and α-glucosidase. Inhibitors of these enzymes delay carbohydrate digestion, causing a reduction in the rate of glucose absorption and consequently blunting glucose rise. Inhibitors of intestinal α-glucosidase have been used in the treatment of non insulin-dependent *diabetes mellitus* (NIDDM) and represented at the huge proportion of anti-diabetic drug market

CONCLUSION

In this investigation, we tested for antimicrobial activity along with α-amylase and α-glucosidase inhibitory effect of extracts of C*. infortunatum* leaves. Based on these results, *C. infortunatum*

can be considered as a potential source of pharmaceutical agent. Further research in *C. infortunatum* will help to analyze therapeutic efficacy of products. Efforts are now being made to investigate various therapeutic actions of *C. infortunatum* plant and their products using model systems.

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