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Research Article

Assessment of *In vitro* anti-inflammatory activity, phytochemical analysis and antimicrobial assay of *Tinospora Cordifolia* extracts

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Abstract

Tinospora cordifolia belonging to the family menispermaceae, is a large extensively spreading glabrous, perennial deciduous twiner with succulent stems and papery bark. In modern medicine, *T. cordifolia* is used for the treatment of general weakness, fever, dyspepsia, dysentery. Methanol extracts of *Tinospora cordifolia* leaves was assessed for its anti-inflammatory activity by *invitro* methods, phytochemical analysis and antimicrobial assay. *Invitro* anti-inflammatory activity was evaluated using albumin denaturation, proteinase inhibitory activity at different concentrations. The results showed that methanolic extracts significantly Inhibited albumin denaturation, maximum inhibition for soxhlet extract was observed at 300µg/ml and for cold extract was observed at 200µg/ml. For Proteinase Inhibitory Action, it showed maximum inhibition for soxhlet extract at 200µg/ml and for cold extract at 100µg/ml. Phytochemical screening revealed the presence of alkaloids, tannins, cardiac glycosides carbohydrates. Quantitative analysis revealed that total alkaloid content was found to be 5370mg/ml in soxhlet extract and 3990mg/ml for cold extract, Tannic acid content in soxhlet methanol extract was found to be 0.637 mg/g TAE of extract, Total carbohydrates content in methanol extract (Soxhlet) = 1.014 mg/ml total carbohydrate content in methanol extract (Cold) = 0.610 mg/ml and cardiac glycosides showed the absorbance of 0.06 and transparency was 88 % when measure for soxhlet methanol extract. The antibacterial assay of the extracts was studied using agar well diffusion method against certain isolates. Results suggest that the cold extract showed some activity against tested bacteria whereas, soxhlet extract showed no activity against tested bacteria.

Keywords: *Tinospora cordifolia*, anti-inflammatory, phytochemical analysis, antimicrobial activity.

INTRODUCTION

The World health Organization (WHO) assessed that upto 80% of the individuals still depend primarily on conventional cures such as restorative plants for their solutions. Since the starting of human civilization, plants have been utilized as normal medications. As of late, researchers are appearing incredible intrigued within the advancement of unused drugs from conventional therapeutic plants. India with tremendous bio-diversity and colossal information of antiquated conventional framework of pharmaceutical such as Ayurveda, Siddha, Unani and Amchiand gives a solid base for the utilization of a huge number of plants in common healthcare and common afflictions of individuals (Pandey et al., 2008).

Among the endless library of critical restorative plants, *Tinospora cordifolia* (wild) may be a deciduous climbing bush which has a place to the family Menispermeaceae. The plant family Menispermeaceae comprises almost 70 class and 450 species that are found in tropical regions. It is found all through India conjointly in parts of Sri Lanka, Bangladesh and China (Raghu et al., 2000). The plant is assigned as Rasayan in Ayurveda and is exceptionally well known for building up the safe framework and body's resistance against tainting microorganisms (Tirtha, 2007).

It is a yearly perpetual Ayurvedic plant which is utilized in a few conventional drugs to remedy different infections. The plant is some of the time developed for fancy esteem and is proliferated by cuttings. The takes off bear a great and

nutritious grub for cattle (Khare, 2007). Nearly all parts of the plants are recorded to be valuable in ethnobotanical overviews conducted by ethnobotanists. *T. cordifolia* is detailed to have antispasmodic, anti-inflammatory and antiallergic. The eminent therapeutic properties detailed are anti-diabetic, anti-periodic, anti-spasmodic, anti-inflammatory, anti-arthritic, anti-oxidant, anti-stress, anti-leprotic, anti-malarial, antipyretic, hepatoprotective, immunomodulatory and anti-neoplastic exercises (Pradhan et al., 2013). The chemical constituents detailed from this bush have a place to distinctive classes such as alkaloids, terpenoids, lactones, glycosides, steroids, sesquiterpenoid, phenolics, aliphatics compounds and polysaccharide. Guduchi satva is a starch obtained from the stem is profoundly nutritive and digestive and used in numerous illnesses. It could be a tonic and valuable within the treatment of constant loose bowels and diarrhea.

The different pharmacological actions of *T. cordifolia* like other medicinal plants can be attributed to the presence of array of secondary metabolites in it (alkaloids, flavonoids, phenols, steroids, saponins, glycosides, etc.)

The previous attempts on anti-inflammatory activity of methanol extract of whole plant are done. *Enicostemma axillare (*Family: Gentianaceae) was assessed for its anti-inflammatory activity by invitro methods. Invitro anti-inflammatory activity was evaluated using albumin denaturation assay, proteinase inhibitory activity, membrane stabilization, and anti-lipoxygenase activity at different concentrations. Aspirin, Diclofenac sodium Indomethacin were used as standard drugs. The results showed that *Enicostemma axillare* Methanol Extract (EAME) at a concentration range of 100-500µg/ml significantly ($p<0.01$) protects the heat induced protein denaturation. At the concentration of 400 and 500µg/ml, EAME showed significant ($p<0.01$) inhibition of42 and 53% of proteinase inhibitory action, but at the concentration of 100 and 200 µg/ml did not show significant ($p>0.05$) activity. Heat induced haemolysis of erythrocyte was significantly ($p<0.05$) inhibited at the concentration of 400 and 500µg/ml. Hypotonicity induced haemolysis and lipoxygenase activity were significantly ($p<0.01$) inhibited at the concentration range of 200-500µg/ml and 400, 500µg/ml respectively. The results obtained in the study indicate that methanol extracts of *Enicostemma axillare* can be a potential source of anti-inflammatory agents (Leelaprakash & Mohan Dass, 2011).

Another study based on analgesic and anti-inflammatory activity (by both in-vitro and in-vivo) of both chloroform and methanol root extracts of *Andrographis serpyllifolia* is done. Methods used for the studies were In-vitro 5-Lipoxygenase inhibition assay and *In-vivo* measurement of rat paw edema and ear edema in rats, acetic acid induced writhing response and hot plate method in albino mice. Chloroform and methanolic extracts of *A. serpyllifolia* root have shown moderate potency in inhibiting 5-LOX and shown significant anti-inflammatory activity. Despite the IC50 values are little higher, anti-inflammatory efficacy of these extracts possibly due to other mechanisms apart of 5-LOX inhibition. However, *In-vivo* anti-inflammatory studies revealed that *A. serpyllifolia* methanolic extract has shown higher degree of efficacy when compared to the chloroform extract. In terms of analgesic activity in writhing test, methanolic extract has shown more efficacy than chloroform extract. Therefore, it is important to isolate the active principles for further testing the anti-inflammatory efficacy (Kandati et al., 2012).

The antibacterial activity of the aqueous, ethanol and chloroform extracts from the stems of *Tinospora cordifolia* was studied using disc diffusion method against Escherichia coli, Proteus vulgaris*, Enterobacter faecalis, Salmonella typhi* (Gram-negative), *Staphylococcus aureus* and *Serratia marcesenses* (Gram-positive) is carried out previously. Results suggested that the ethanolic extract has significant antibacterial activity against tested bacteria. The study justifies the claimed uses of *Tinospora cordifolia* in the traditional system of medicine to treat various infectious diseases (Jayachandran et al., 2003). The study was designed to investigate antioxidant properties (through invitro method) as well as brine shrimp lethality and phytochemical group evaluation of stem part of *Z. rugosa* Lam extracted with different solvents i.e., from nonpolar to polar (petroleum ether > ethyl acetate > ethanol > methanol > water). Phytochemical investigation showed the presence of alkaloids, flavonoid, glycosides and carbohydrates which provides evidence on good to moderate antioxidant and good lethality properties of the subjected plant (Gracelin et al., 2013). Ethyl acetate extract of stem was found to contain the highest number of phenols (97.188 ±12.816 mg/g gallic acid equivalent) and flavonoids (15.009 ± 0.385 mg/g quercetin equivalent). In 2, 2-diphenyl, 1-picrylhydrazyl (DPPH) free radical scavenging assay, among all the extracts ethanolic stem extract showed the highest scavenging property. Whereas standard drug ascorbic acid showed. But in nitric oxide scavenging assay maximum scavenging of nitric oxide was found with water extract of stem comparatively similar to standard ascorbic acid. Methanolic stem extract was found to contain the greater reducing power in reducing power capacity assessment (correlation coefficient r =0.99 and P\0.001).

Previously the antioxidant potential of solvent extracts of leaf and stem of *T. cordifolia* were evaluated by various invitro methods. Scavenging effects on DPPH, ABTS radical, hydroxyl radical and ferric reducing antioxidant power (FRAP) were found to be highest in methanolic extract of leaf and ethyl acetate extract of stem compared to all other extracts. These extracts also exhibited significant protection against radical induced protein (BSA) oxidation and plasmid DNA damage (pBR322). The extracts were further evaluated for their inhibitory properties on AAPH (2, 2'-azo (2-amidinopropane) dihydrochloride induced ex vivo oxidative stress in rat liver homogenates. The results showed the potent antioxidant nature of methanolic extract of leaf

and ethyl acetate extract of stem with respect to inhibition of lipid and protein oxidation. Overall, stem extracts showed to be the more effective antioxidant source than the leaf extracts with regard to all the radical scavenging activities (Ugochukwu et al., 2013). These protective properties of the extracts could be directly attributed to the presence of phytochemicals such as polyphenols, tannins etc.

The study was carried out to analyze the phytochemical compounds in leaves and stem extracts of *T. cordifolia* by using phytochemical screening tests and estimate total flavonoid content (TFC) by using aluminum chloride method in the sample extracts. The leaf and stem extracts of *T. cordifolia* expressed the presence of several phytochemicals viz., flavonoids, amino acids, diterpines, protein, saponins and carbohydrates Oyedepo and Femurewa (1995). The result of phytochemical screening tests revealed that diterpines and carbohydrates are positive in all extracts of *T. cordifolia*, but flavonoids and saponins only present in methanol and ethanol extracts. TFC of *T. cordifolia* was higher in ethanolic leaves extracts than methanolic leaves extracts. These studies justify that *T. cordifolia* use in traditional medicines. The investigation further proposed that the phytochemicals present in stems and leaves of *T. cordifolia*, which can be use as natural antioxidants in medicinal drugs Garg and Garg (2018).

MATERIALS AND METHODS

Plant material

The whole plants of Tinospora Cordifolia were collected in fresh condition from Nagpur, Maharashtra. The plant was shade dried then ground in to a uniform powder using a blender and stored in polythene bags at room temperature.

Preparation of extract using soxhlet

The plant powder was stacked in to soxhlet extractor and subjected to extraction with methanol. After extraction, the solvent was refined off and the extracts were concentrated on water bath to a dry residue and kept in a desiccator (Figure 1).

Preparation of extract using cold extraction method

In this process, the coarsely powdered plant sample is placed in a flat bottom flask with the solvent and allowed to stand at room temperature for a period of 2-3 days on a magnetic stirrer with continuous agitation until the soluble matter has dissolved. The mixture then is strained, the marc (the damp solid material) is pressed, and the combined liquid is clarified by filtration or decantation after standing (Sazzad et al., 2013).

Assessment of *in-vitro* anti-inflammatory activity

Inhibition of albumin denaturation:

The anti-inflammatory activity was studied by using inhibition of albumin denaturation technique which was studied according to (Mizushima & Kobayashi, 1968; Sakat et al., 2010). The reaction mixture (0.5ml) consisted of 0.45ml bovine serum albumin (5% aqueous solution) and 0.05ml of sample extracts (200 and 400µg/ml). pH was adjusted at 6.3 using a small amount of 1N HCl. The samples were incubated at 37°C for 20minutes and then heated at 57°C for 3 minutes. After cooling the samples, 2.5ml phosphate buffer saline (pH 6.3) was added to each tube. Turbidity was measured spectrophotometrically at 660nm. For control tests 0.05ml distilled water was used instead of extracts while product control tests lacked bovine serum albumin. The percentage inhibition of protein denaturation was calculated as follows:

Percentage inhibition = (Abs Control-Abs Sample) X 100/ Abs control.

The control represents 100% protein denaturation. The results were compared with Aspirin (100µg/ml) treated samples.

Ant proteinase action

The test was performed according to the modified method of Oyedepo *et al* and Sakat *et al*. The reaction mixtures (2.0ml) contained 0.06 mg trypsin, 1.0ml 25mM tris-HCl buffer (pH 7.4) and 1.0ml aqueous solutions of sample extract (200 and 400µg/ml). The mixtures were incubated at 37° C for 5minutes. Then 1.0 ml of 0.8% (w/v) casein was added. The mixtures were incubated for an additional 20 minutes. 2.0 ml of 70% (v/v) perchloric acid was added to terminate the reaction. The cloudy suspension was centrifuged. Absorbance of the supernatant was read at 280nm against buffer as blank. The percentage of inhibition was calculated. Aspirin (100µg/ml) was used as standard.

Percentage inhibition = (Abs control-Abs sample) X 100/ Abs control.

Phytochemical analysis

Qualitative analysis:

The preliminary phytochemical analysis was used to analyse the presence of compounds namely Alkaloids, Flavonoids, Saponins, Tannins, Phenols, Proteins, Cardiac glycosides, Terpenoids, Carbohydrate and Quinones.

Quantitative analysis:

Depending on above qualitative results the quantitative assay is carried out for Alkaloids, Tannins, Carbohydrates and cardiac glycosides.

Antimicrobial assay:

Antimicrobial activity of *Tinospora cordifolia* leaf extracts was carried out using agar well diffusion method against *Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Salmonella sp., Chromobacterium sp.* This bacterium's was cultured in nutrient broth for 24 hours at 37ºC.

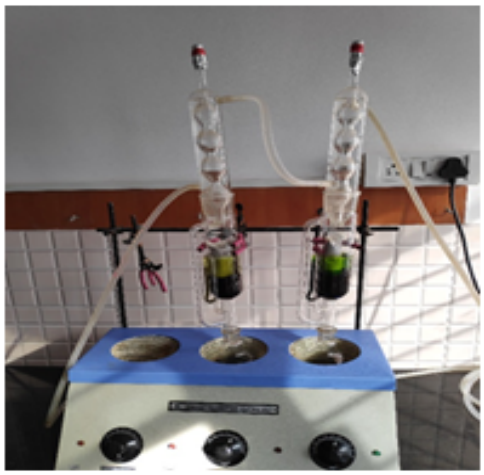

Figure 1: Soxhlet assembly.

Agar plate was prepared by adding 28ml of MHA media into the sterile petri plate, allowed to solidify and keep in the incubator for 24 hours to check contamination.

RESLUTS AND DISCUSSION

Assessment of *in-vitro* anti-inflammatory activity

Inhibition of albumin denaturation:

Protein Denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat. Most biological proteins lose their biological function when denatured. Denaturation of proteins is a well-documented cause of inflammation. As part of the investigation on the mechanism of the anti-inflammation activity, ability of plant extracts to inhibit protein denaturation was studied. It was effective in inhibiting heat induced albumin denaturation. Maximum inhibition of 98.1% (for soxhlet extract) was observed at 300µg/ml and 99.4% (for cold extract) was observed at 200µg/ml. Aspirin, a standard anti-inflammation drug showed the maximum inhibition 75% at the concentration of 100 µg/ml compared with control (Figure 2 & 3).

Soxhel extract Figure 2:

Cold extract Figure 3:

Proteinase inhibitory action:

Neutrophils are known to be a rich source of serine proteinase and are localized at lysosomes. It was previously reported that leukocytes proteinase plays an important role in the development of tissue damage during inflammatory reactions and significant level of protection was provided by proteinase inhibitors. TCME exhibited significant Antiproteinase activity at different concentrations as shown in Table 1. It shows maximum inhibition of 97.83% (for soxhlet extract) was observed at 200µg/ml and 98.57% (for cold extract) was observed at 100µg/ml. Aspirin, a standard anti-inflammation drug showed the maximum inhibition 75% at the concentration of 100 µg/ml compared with control (Figure 4-7).

Soxhlet extract Figure 4:

Cold extract Figure 5:

Phytochemical screening of soxhlet extract and cold extract *Tinospora Cordifolia* leaves

Qualitative phytochemical analysis Table 1:

Quantitative phytochemical analysis:

Total alkaloid content Figure 6:

Total tannin content Figure 7:

Total cardiac glycosides content:

The OD was taken at 495nm and *Securigera securidaca* used as standard.

The absorbance of the sample extract (Soxhlet) was found to be 0.06 and percent transparency was 88 (Figure 8).

Total carbohydrates content Figure 8:

Antimicrobial assay of methanolic extracts of *tinospora cordifolia* using agar well diffusion method

Results of antimicrobial assay of the leaves extracts of *Tinospora cordifolia* were measured in terms of inhibition. The zones of inhibition in diameter (mm) recorded for soxhlet extract and cold extract are depicted in Table 2. It is revealed that the cold extracts exhibited significant antibacterial activity against *Bacillus subtilis* and *Chromobacterium sp.* and moderate activity was observed against *Salmonella sp.* and showed no activity against *Escherichia coli* and *Staphylococcus aureus*. However, the soxhlet extract showed no activity against any of the bacterium (Figures 9-14).

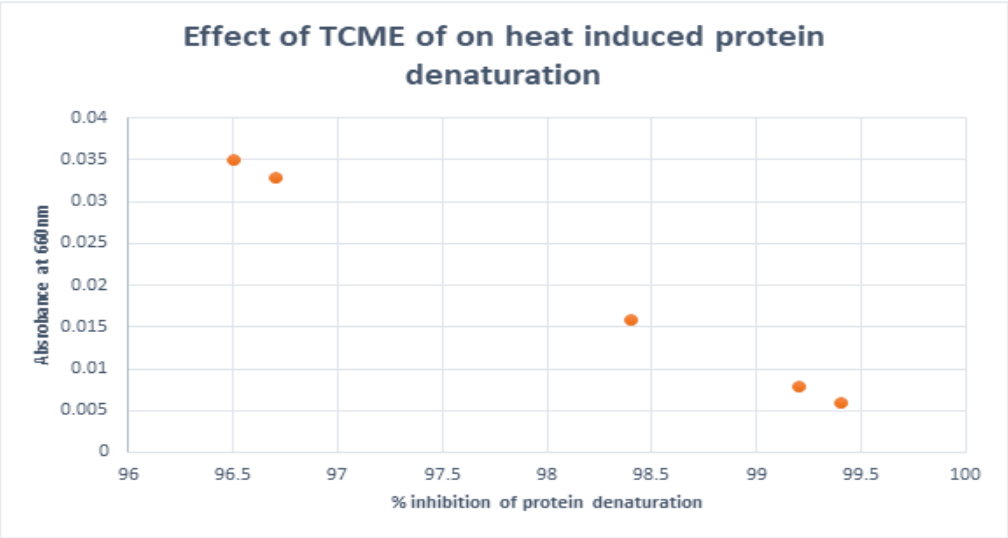

Figure 2: Effect of TCME on heat induced protein denaturation.

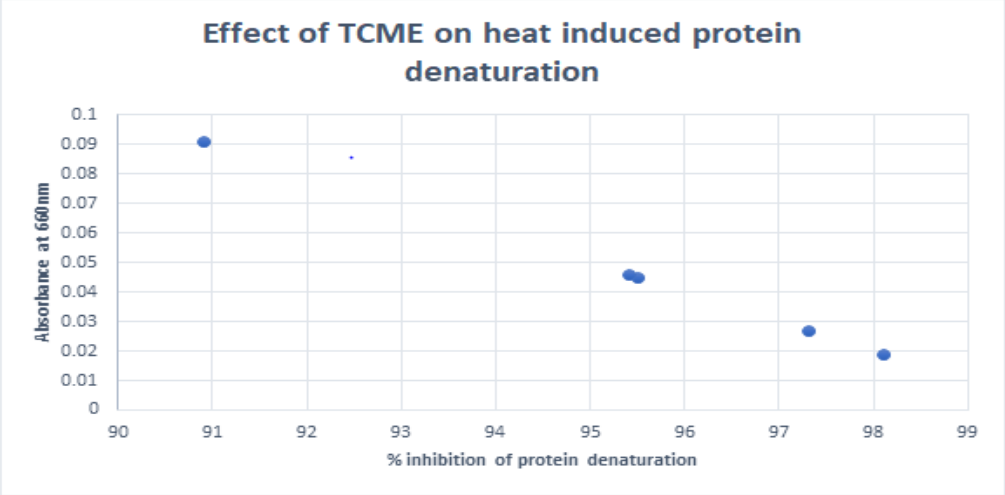

Figure 3: Effect of TCME on heat induced protein denaturation.

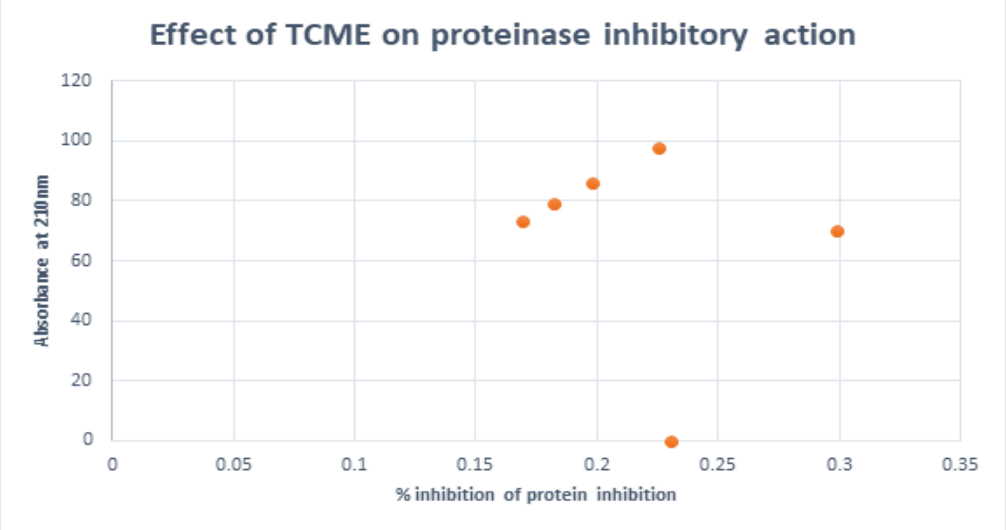

Figure 4: Effect of TCME on proteinase inhibitory action.

Table 1. Qualitative phytochemical analysis.

Phytochemical Constituents	Methanol Extract (SOXHLET)	Methanol Extract (COLD)
Alkaloids	+	+
Flavonoids	-	-
Saponins	-	-
Tannins	+	-
Phenols	-	-
Proteins	-	-
Cardiac glycosides	-	+
Terpenoids	-	-
Carbohydrates	+	+
Quinones	-	-

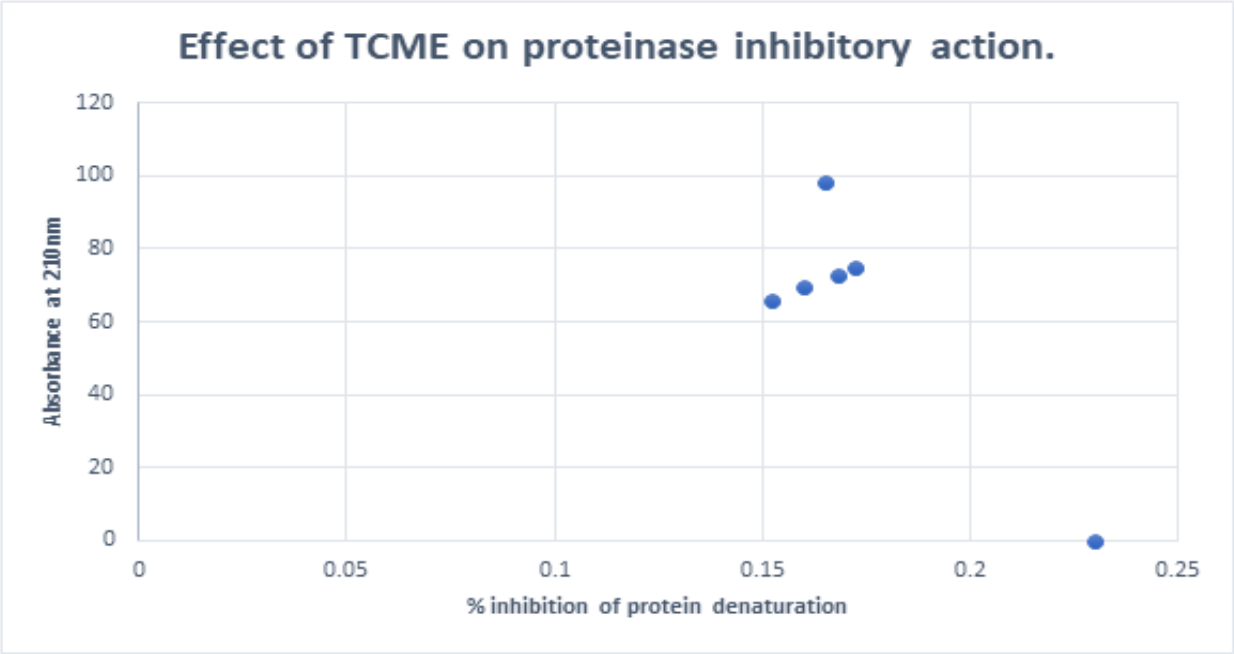

Figure 5: Effect of TCME on proteinase inhibitory action.

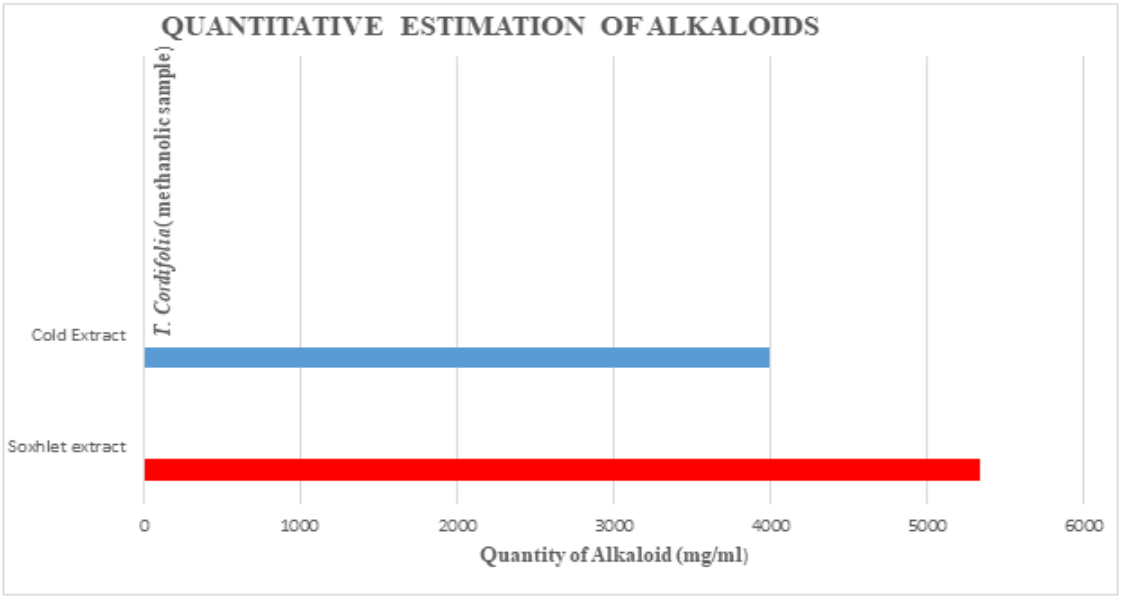

Figure 6: Total Alkaloids content estimation in *T. Cordifolia* sample extracts.

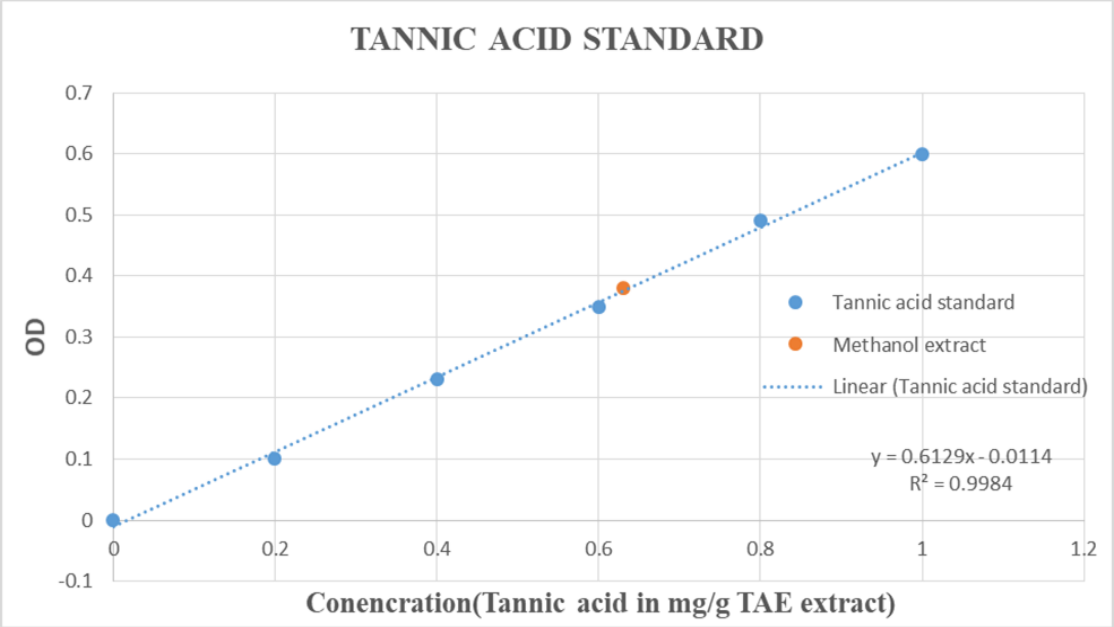

Figure 7: Total Tannin content estimation in *T. Cordifolia* sample extracts.

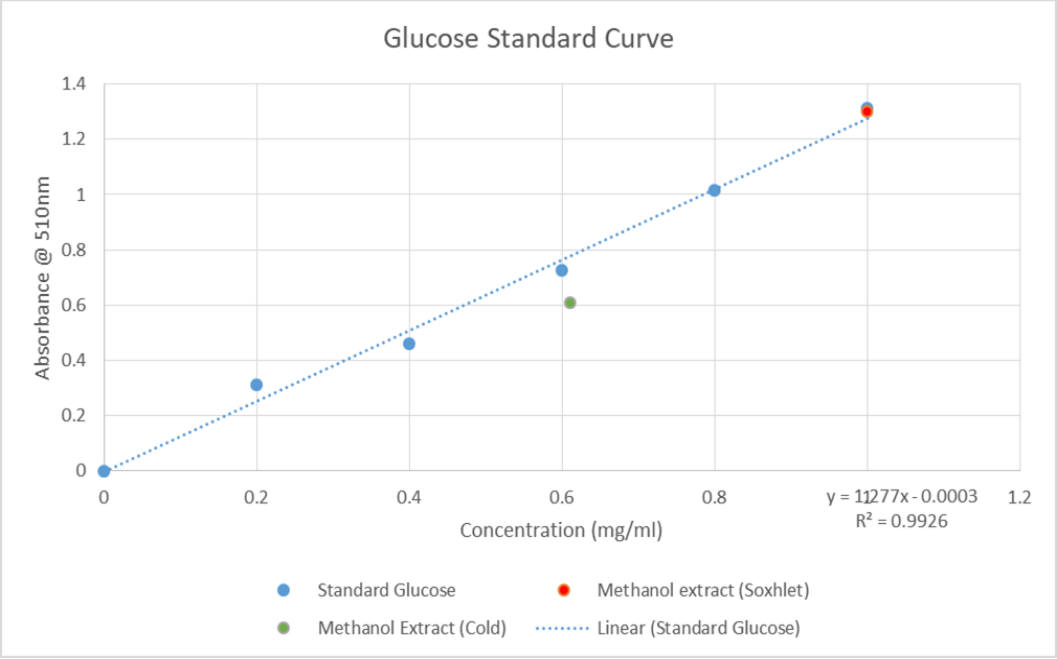

Figure 8: Total Carbohydrates content estimation in *T. Cordifolia* sample extracts.

Table 2: Test Organisms.

Test Organisms	Methanolic Extract	Inhibition Zones (mm)
Bacillus subtilis	Cold	12
	Soxhlet	-
Chromobacterium sp.	Cold	10
	Soxhlet	-
Salmonella sp.	Cold	7
	Soxhlet	-
Escherichia coli	Cold	-
	Soxhlet	-
Staphylococcus aureus	Cold	-
	Soxhlet	-

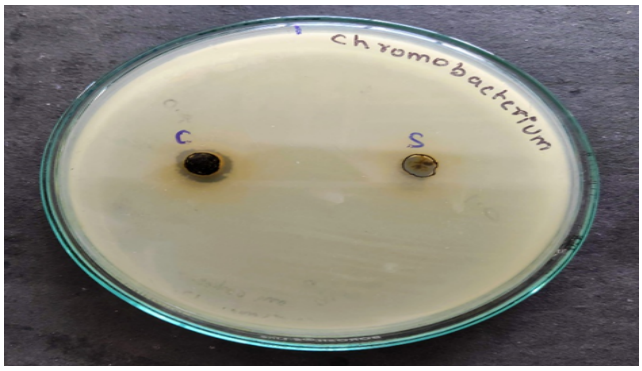

Figure 9: Activity against *Chromobacterium sp*.

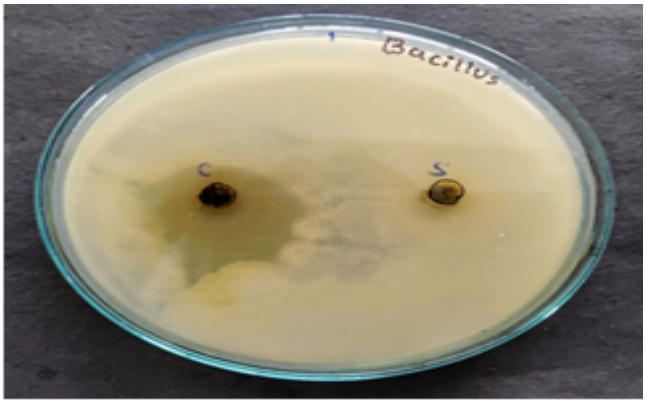

Figure 10: Activity against *Bacillus subtilis.*

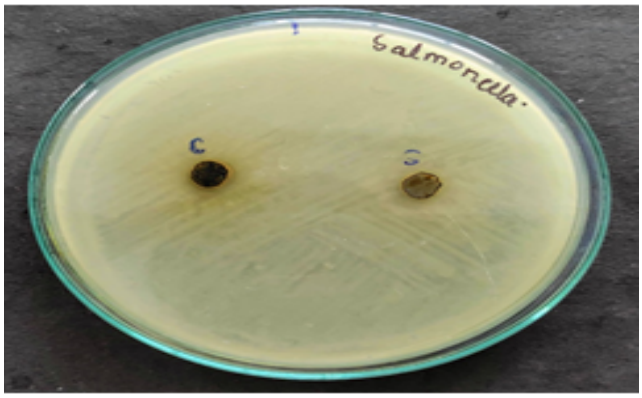

Figure 11: Activity against *Salmonella sp*.

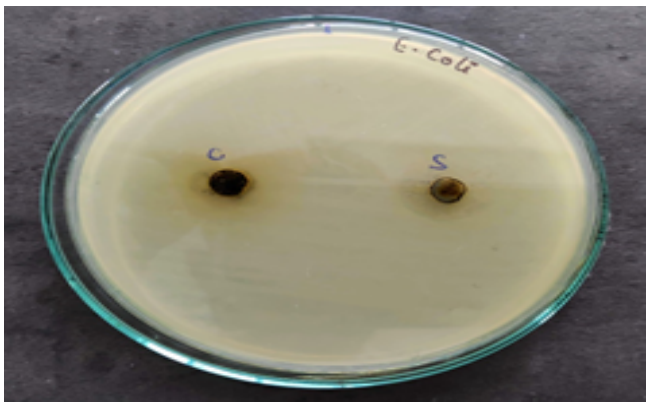

Figure 12: Activity against *Escherichia coli*.

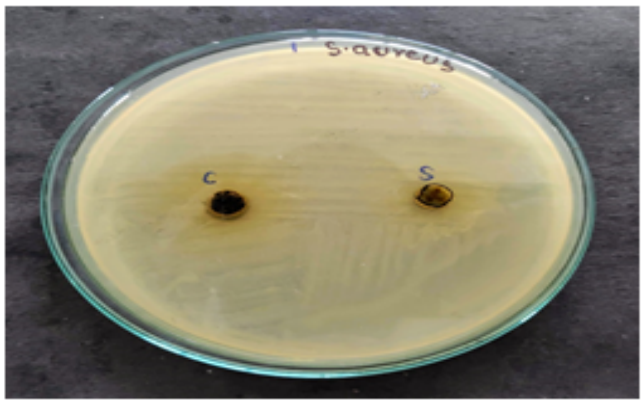

Figure 13: Activity against *Staphylococcus aureus.*

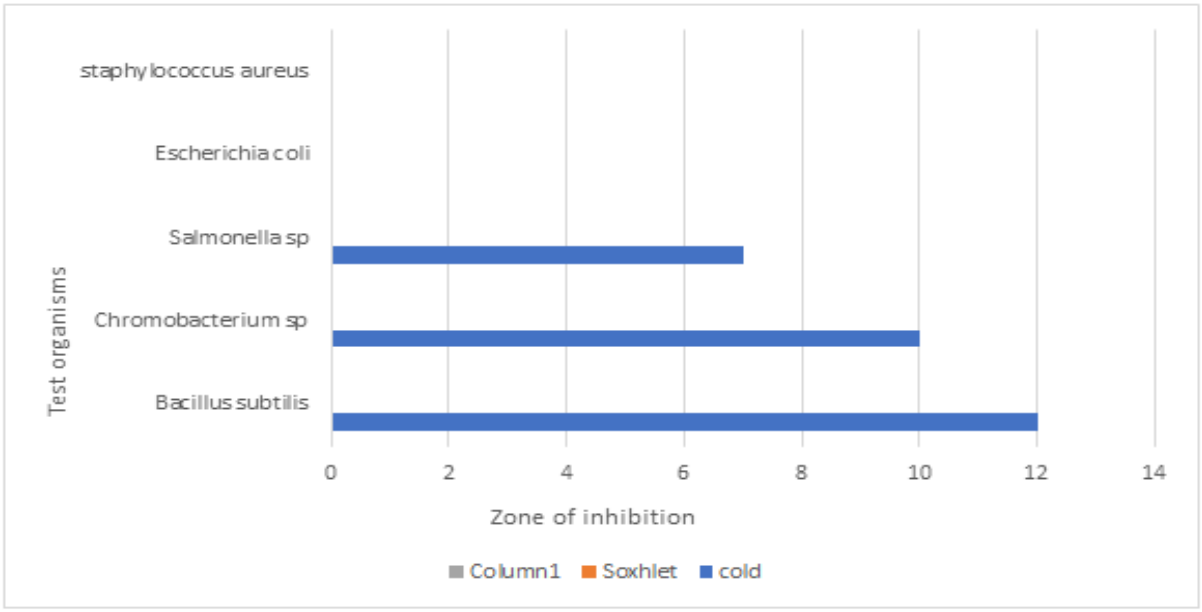

Figure 14: Test Organisms.

CONCLUSION

The methanol extracts of *Tinospora cordifolia* possesses anti-inflammatory properties. These activities may be due to the strong occurrence of polyphenolic compounds such as alkaloids, flavonoids, tannins, steroids, and phenols, The extract fractions served as free radical inhibitors or scavenger or acting possibly as primary oxidants and inhibited the heat induced albumin denaturation, proteinase activity. TCME also reduced the activity of lipoxygenase. Purification of each bioactive compound is necessary and this purified form of the compound can be used which may show increased activity. This gives an idea that the compound of the plant *Tinospora cordifolia* can be used as lead compound for designing a potent anti-inflammatory drug which can be used for treatment of various diseases such as cancer, neurological disorder, aging and inflammation.

Phytochemical screening and analysis will be useful in the presence and quantification of the bioa4ctive principles and subsequently may lead to the drug discovery and development. This study revealed the presence of medicinally important constituents in the studied species. Many evidences gathered in earlier studies also confirm the identified phytochemicals to be bioactive.

Therefore, extracts from these plants can be used as a good source for useful drugs and their quantified values can be used as a major tool for obtaining a quality control profile for a drug.

Plant extracts contained a very complex structure with the active ingredients present in the form of natural organic compounds. The process of extraction for a particular compound is dependent on the solubility of the component in the solvent. The process and extraction system are constantly different with every product and compound. Most of the phytochemical compounds are absent in this particular solvent extraction for this plant hence cold shown very less activity and Soxhlet process have shown negative antimicrobial activity. The potential to develop antimicrobial compounds from higher plants appears rewarding as it will propel to the expansion of a phytomedicine to turn against multidrug resistant microbes.

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