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Original Article

ANTIBACTERIAL, ANTIOXIDANT AND ANTIPROLIFERATIVE ACTIVITIES OF SOLVENT EXTRACTS OF *TILIACORA ACUMINATA*

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ABSTRACT

Objective: To study the antibacterial, antioxidant and anti proliferative activities of ethanol, methanol and ethyl acetate extracts of *Tiliacora acuminata* leaves

Methods: Phytochemical tests were performed according to the protocols described by Matos. Total phenolic content, total flavonoid content, Gas Chromatography-Mass Spectrometry (GC-MS) analysis, Fourier Transform Infrared Spectrophotometer (FT-IR) analysis, DPPH radical scavenging activity, Hydrogen peroxide scavenging activity, antimicrobial test, brine shrimp lethality test and cytotoxicity assay were performed according to standard methods.

Results: Ethyl acetate extracts showed the presence of the higher amount of phenolics (320.52 gallic acid equivalent/gm dry weight and flavonoids (250.06 quercetin/gm dry wt). GC-MS analysis showed the presence of alpha.-Tocopherol-beta.-D-mannoside, n-Hexatriacontane and Neophytadiene. Ethyl acetate extract showed higher zone of inhibitions with *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella typhimurium* and *Klebsiella pneumonia.* The extract also showed cytotoxic activity in human laryngeal cell line and a study on brine shrimps showed positive effect of cytotoxicity with LC_{50} of 85µg. Further analysis of leaf extract by GC-MS and FTIR proved the presence of various phenolics and flavonoids.

Conclusion: Ethyl acetate extracts of *Tiliacora acuminata* showed promising antibacterial, antioxidant and cytotoxic activities. This study may be very much helpful for the development of some new drugs based on *Tiliacora acuminata* in future.

Keywords: *Tiliacora acuminata*, Antibacterial, Antioxidant, Cytotoxicity, GC-MS, -, *T. Acuminate*

INTRODUCTION

Plants have been valuable and indispensable sources of natural products for the health of human beings and they have a great potential for producing new drugs [1-3]. Even today people who live near to forest use plant products to cure chronic diseases. Tropical and subtropical areas of the world are bestowed with abundant herbs and flora which have untapped properties, such as antimicrobial, antioxidant and anticancer activities. Nowadays there is widespread interest in evaluating drugs derived from plant sources. This interest primarily stems from the belief that green medicine is safe and dependable compared to costly synthetic drugs which are invariably associated with adverse effects. The adverse effects of the drugs available today, necessitate the discussion of new and harmless pharmacotherapeutic agent from medicinal plants [4].

Plants produce a wide array of secondary metabolites such as phenolic compounds (phenolic acids, flavonoids, quinines and coumarins), nitrogen compounds (alkaloids and amines), vitamins, terpenoids and other secondary metabolites that have been proven as antimicrobial, antioxidant and antineoplastic agents. Recent investigations have confirmed that antioxidants are the most effective tools to eliminate free radicals which cause oxidative stress and act as possible protective agents that protect the cells from reactive oxygen species (ROS) and retard the progress of many diseases [5-7]. Moreover, in the recent past, the polyphenols have been found to be beneficial as strong antioxidants [8]. In this context, evaluation of the polyphenols and their antioxidant activities in herbs has become an important tool to understand the healing property of medicinal plants.

According to the World Health Organization (WHO), nearly 20,000 medicinal plants exist in 91 countries including 12 mega biodiversity countries. The premier steps to utilize the biologically active compound from plant resources are extraction, pharmacological screening, isolation and characterization of bioactive compound, toxicological and clinical evaluations. Keeping in view of the demand for developing natural antioxidants, antiproliferative and antimicrobial drugs, the present study was undertaken to investigate the antioxidant, free radical scavenging, antimicrobial and antiproliferative activities of ethanol, methanol and ethyl acetate extracts of *T. acuminata* which belongs to Family Menispermaceae. This plant was chosen for this study based on its traditional usage. A survey of the literature revealed that the whole plant is an antipyretic, alleviates spasms and stimulates the cardiovascular and central nervous systems and also used for casting out snakes poison [9].

The seeds also serve as cardiac tonic, aphrodisiac, diuretic, antispasmodic. Based on these properties, present investigation was taken to evaluate the antioxidant, antiproliferative and antibacterial potentials of this plant, in addition to analyzing the polyphenols which might be responsible for biological activities. In order to get some basic molecular data, FTIR and GC-MS were also performed on the extracts of *T. acuminata.* This study will be very much helpful for the development of any plant based drugs in the future.

MATERIALS AND METHODS

Chemicals

All the chemicals were purchased from Hi-Media and Merck, India. Standard drugs were purchased from Sigma-Aldrich chemicals co. All the solvents used in this study are analytical grade solvents.

Microbial cultures

Bacterial reference strains used in this study were *Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella typhimurium, Klebsiella pneumonia*; these were obtained

from Kings Institute, Chennai, India. The pure cultures were maintained on nutrient agar slants for the entire study. All the isolates were sub-cultured at regular intervals and stored at 4°C as well as at -80°C (glycerol stocks).

Plant material and solvent extraction

Healthy leaves of *T. acuminata* were collected from vacant plots in Chennai, India. The leaves were cleaned, dried under the shade, ground to a coarse powder and stored in an airtight container at 25°C for further use. Extraction was performed on a soxhlet apparatus using 10g of sample with ethanol (99%), methanol (absolute), or ethyl acetate (99%) as solvents for 8 hours till clear colorless solvent was obtained indicating that no more extraction from the plant material was possible. The extract was concentrated to the dry mass using vacuum evaporator. The residues were stored in amber glass bottles at -20°C for further analysis. The dried extracts of stock solution were prepared and different concentrations were used in the experiments.

Phytochemical analysis of plant extracts

Phytochemical tests were performed following the protocols described by Matos [10], based on reactions with specific reagents for the main classes of natural products with precipitate formation or color change.

Determination of total phenolic content

The amount of total soluble phenolic content in different extracts was determined according to Folin-Ciocalteu method [11] with slight modifications. Briefly, 10 µL of extract solution was mixed with 100 µL of Folin-Ciocalteu reagent. After 10 min of incubation, 300 µL of 20% Na_2CO_3 solution was added and the volume was made up to 1 mL using distilled water. The mixture was incubated in dark for 2h and the absorbance was measured at 765 nm using a UV-Visible spectrophotometer (UV-2600, Shimadzu, Japan) against blank sample. The total phenolic content was measured as Gallic acid equivalents (mg GAE)/gram dry weight (dw) and the values were presented as means of triplicate analysis.

Determination of total flavonoid content

Total flavonoid content was estimated by colorimetric method [12] by taking 20 µL of each extract and mixed with 500 µL double distilled water and 30 µL of 5% $NaNO_2$ solution. After incubation for 5 min at room temperature, 60 µL of 10% $AlCl_3$ solution was added. Subsequently, 350 µL of 1 M NaOH and 40 µL of double distilled water were added to make the final volume to 1 mL. Samples were further incubated for 15 min at room temperature and the absorbance was measured at 510 nm. The total flavonoids were determined as qurecetin equivalents (mg QE)/g of dry wt and the values were expressed as the means of triplicate.

Gas chromatography-mass spectrometry (GC-MS) analysis

The GC-MS analyses of the ethyl acetate extract were conducted with GC-MS-QP 2010 [Shimadzu, Japan]. The Vf -5ms column was used and Helium was used as the carrier gas with a flow rate of 1 µl per minute. Plant extract was injected into the system in split mode at 240°C. The column oven temperature was maintained at 110°C for 3 minutes, then programmed at 75°C to 300°C for 1 minute and increased to 280°C by sequential increment of 5°C per minute. The compounds were identified using the database available in the literature in the journals and books.

Fourier transform infrared spectrophotometer (FT-IR)

Dried powder of methanolic extract was considered for FT-IR analysis (Shimadzu, IR Affinity 1, Japan). For the FT-IR study, dried powder of methanolic extract was ground finely with KBr and the translucent disc was prepared using QwikHandi press (details of the instrument, make, model, country, etc.). The powdered samples of plant extracts were treated for FTIR spectroscopy. Scan range: from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1} was used. The result was analyzed based on peak obtained

DPPH radical scavenging activity

The antioxidant activity of the leaf extracts was assessed on the basis of the radical scavenging effect using stable 1,1-diphenyl-2-picryl hydrazyl (DPPH) [13]. DPPH solution (0.004% w/v) was prepared in 95% methanol and serial dilutions was carried out with the stock solutions of the extracts (20-200µg). Various concentrations of extracts were mixed with DPPH solution (900 µL), incubated in the dark for 30 min and then absorbance was measured at 517 nm. Methanol (95%), DPPH solution and ascorbic acid (AA) were used as blank, control and reference standard respectively and IC_{50} was calculated.

Hydrogen peroxide scavenging activity

Scavenging activity of hydrogen peroxide was determined by Ruch et al. Method [14]. Plant extracts (4 ml) prepared at various concentrations (20-200µg) in distilled water was mixed with 0.6 ml of 4 mM H_2O_2 solution which was prepared in phosphate buffer (0.1 M, pH 7.4) and incubated for 10 min. The absorbance of the solution was measured at 230 nm against the blank solution containing the plant extract without H_2O_2 and IC_{50} was calculated.

Preliminary antimicrobial test

Preliminary antimicrobial screening was performed using the agar well diffusion bioassay. Tubes containing 5 ml Mueller-Hinton broth (MHB) were inoculated from an overnight culture of bacterial strains. After 3 h of incubation, 100 µl of each microorganism (approximately 10^6 CFU) was spread separately onto the surface of muller hinton agar [13]. Wells was made in the agar by using an inverted sterile Durham tube (6 mm in diameter), and 100 µl (100µg) of each extract was deposited in the well. Plates were incubated at 37°C for 24 h. Antimicrobial activity was detected by the presence of a growth inhibition zone surrounding the well. Diameter of the zone was measured and recorded. Solvents used for each extract were employed as controls.

Brine shrimp lethality test

Dried cysts were incubated (1 g cyst per liter) in a hatcher at 28-30°C with strong aeration, under a continuous light regime [15]. Approximately 12 h after hatching, the phototropic nauplii were collected with a pipette from the lighter side and concentrated in a small vial. Ten brine shrimps were transferred to each well using adequate pipette. Each test consisted of exposing groups of 10 Artemia aged 12 h to various concentrations of the toxic compound (10-200 µg). The toxicity was determined after 12, 24 and 48 h of exposure. The number of survivors was counted and percentages of death were calculated. Larvae were considered dead if they did not exhibit any internal or external movement during several seconds of observation.

To ensure that the mortality observed in the bioassay could be attributed to bioactive compounds and not to starvation; we compared the dead larvae in each treatment to the dead larvae in the control. In any case, hatched brine shrimp nauplii can survive for up to 48 h without food [16] because they still feed on their yolk-sac [17]. However, in cases where control deaths were detected, the percentage of mortality (% M) was calculated as: % M = percentage of survival in the control - percentage of survival in the treatment.

Cytotoxicity assay

Cell viability was evaluated by the MTT method (Sigma-Aldrich, USA) using 3-(4, 5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide [18,19]. The cytotoxicity of the extracts was assessed employing human HEp 2 (laryngeal carcinoma) cell lines. The monolayer cell culture was trypsinized and the cell count was adjusted to 3-lakhcells/ml using the medium containing 10% newborn calf serum. To each well of 96 well microtitre plates, 0.1 ml of diluted cell suspension was added. After 24 hours, the monolayer formed the supernatant which was flicked off and 100 µl of different test compounds (20-200µg) were added to the cells in microtitre plates and incubated at 37ºC in 5 % CO_2 incubator for 72 hours and cells were periodically checked for granularity, shrinkage, swelling. After 72 hours, the sample solution in wells was flicked off and 50µl of MTT dye was added to each well. The plates were gently shaken and incubated for 4 hours at 37°C in 5% CO_2 incubator. The supernatant was removed, 50 µl of DMSO was added, and the plates were gently shaken to solubilize the formed formazan. The

absorbance was measured using a microplate reader at a wavelength of 490 nm. The percentage growth inhibition was calculated using the following formula

Percentage of cell inhibition= 100-{(At-Ab)/ (Ac-Ab)} x100

Where,

At= Absorbance value of test compound

Ab= Absorbance value of blank

Ac=Absorbance value of control.

Table 1: Details on various phytochemicals present in *Tiliacora acuminata* solvent extracts

Name of the Phytochemicals	Name of the test	Name of the solvent extract		
		ethanol	Methanol	Ethyl acetate
Alkaloids	Hager's test	++	+ +	+++
Reducing sugars	Fehling's test	+	+	+
Saponins	Frothing test	+	+	+
Tannins	$FeCl_3$ test	+	++	+ ++
Flavonoids	Ammonia test	+	+ +	+ ++
Anthraquinones	Chloroform layer test	+	+	+
Cardiac glycosides	Killer kilani's test	+	+	++
Terpenoids	Salkowski test	+	++	+++
Steriods	Salkowski test	-	-	+

Table 2: Details of polyphenol and flavonoid content in ethanol, methanol and ethyl acetate extracts of *Tiliacora acuminata*

Type of Extract	Polyphenols (GAE/g dry weight)	Flavonoids (quercetin/g dry weight)
Ethanol	180.10±0.03	100.12±0.13
Methanol	250.68±0.02	120.51±0.10
Ethyl acetate	320.52±0.02	250.06±0.17

GAE = Gallic acid equivalent

Table 3: GC-MS analysis of ethyl acetate extract of *Tiliacora acuminata*

Peak Number	Retention time	Area %	Molecular name
1	20.676	0.79	8-METHYL-1-DECENE
2	20.757	37.05	NEOPHYTADIENE
3	20.808	3.04	(2E)-3,7,11,15-Tetramethyl-2-hexadecene
4	21.014	7.24	(2E)-3,7,11,15-Tetramethyl-2-hexadecen-1-ol
5	21.203	11.30	3,7,11,15-TETRAMETHYLHEXADEC-2-EN-1-OL
6	21.593	0.65	(5Z)-2,6,10-Trimethyl-1,5,9-undecatriene
7	23.037	2.09	Isopropyl 2-phenyl-4,5-dihydro-1,3-oxazole-4-carboxylate
8	30.959	3.64	n-Hexatriacontane
9	31.278	10.76	alpha.-Tocopherol-. beta.-D-mannoside
10	32.422	3.64	n-Hexatriacontane
11	33.272	4.94	Cholest-4-en-3-oneq
12	33.507	7.32	24(S)-Ethyl-3. alpha.,5. alpha.-cyclocholest-22(E)-en-6-one
13	34.183	7.54	Testosterone cypionate

Table 4: Details of FT-IR peak values and functional groups in ethyl acetate extract of *Tiliacora acuminata*

Peak values	Functional groups
3834.2	Alcohols
3741.6	Alcohols,phenols
2916.1	Aliphatic amines
2360.7	Nitriles
1650.9	Alkenes
1519.7	Aromatics
1164.9	Aliphatic amines
987.48	Amines
771.47	Alkyl halides

RESULTS

Phytochemical analysis

Preliminary phytochemical screening of the plant extracts revealed the presence or absence of various bioactive components like alkaloids, tannins, flavanoids, terpenoids, steroids and saponins (Table 1). Ethyl acetate extract showed the presence of maximum components than the other two extracts.

Total phenolic and flavonoid content

The results of total phenolic and flavonoid content of different extracts of *T. acuminata* are given in Table 2. The total phenolic content in the leaf extracts expressed as gallic acid equivalent (GAE) per gram dry weight of the extract and the flavonoid content expressed as quercetin equivalent per gram dry weight of the extract. Ethyl acetate extracts showed the presence of higher amount of phenolics and flavonoids. There is a significant difference ($p<.005$) in percentage of phenolic and flavonoid content in different plant extracts of *T. acuminata.*

GC-MS analysis

In GC-MS analysis, 13 compounds were identified in the ethyl acetate extract of *T. acuminata.* The identification of phytochemical compounds is based on the peak area, molecular weight and

retention time as presented in Table 3. GC-MS analysis confirmed the presence of Terpenoids alkalene and steroids.

FTIR analysis

The results of FT-IR spectrum (Figure 1) showed the presence of various functional groups like alcohols, phenol, aliphatic amines and aromatic which qualitatively confirm the results of preliminary phytochemical screening as represented in Table 4.

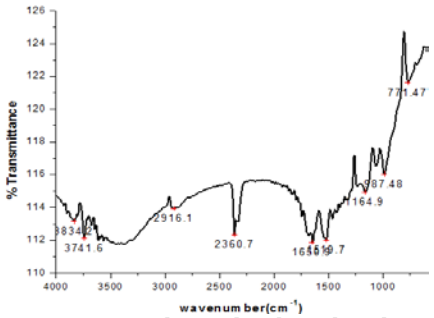

Fig. 1: FTIR spectrum obtained with methanol extract of *Tiliacora acuminate*

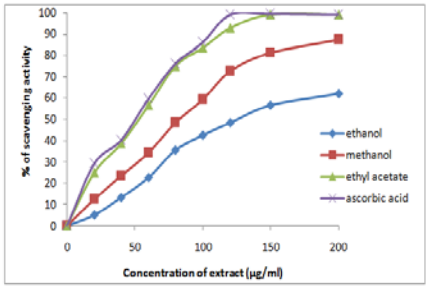

Fig. 2: DPPH scavenging activity of solvents extracts of *Tiliacora acuminata*

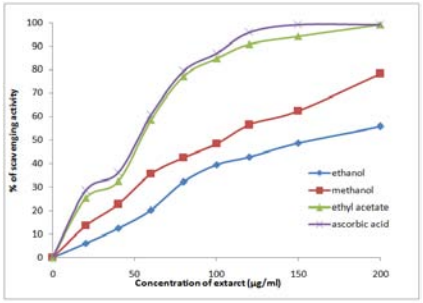

Fig. 3: H_2O_2 radical scavenging activity of solvents extracts of *Tiliacora acuminate*

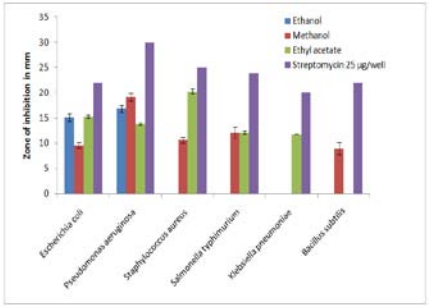

Fig. 4: Antibacterial activity of ethanol, methanol and ethyl acetate extracts of *T. acuminata*. Species is given in X-axis and zone of inhibition (in mm) is given in Y-axis. Values are mean ± SD

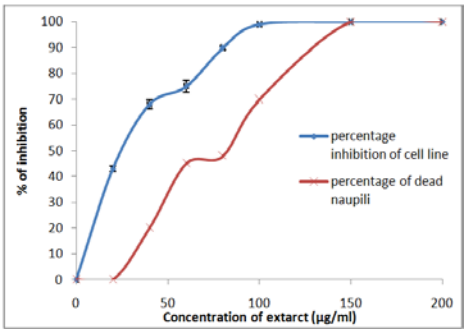

Fig. 5: Brine shrimp and MTT assay of ethyl acetate extract of *T. acuminata*.

DPPH scavenging activity

From the analyses of Figure 2, it can be concluded that the scavenging effect of *T. acuminata* increases with increase in concentration. The results revealed that the ethyl acetate extracts of the leaf had the higher DPPH radical scavenging ability and was close with the ascorbic acid, the standard, followed by methanol and ethanol extracts. The IC_{50} was found to be 158, 106, 52, 50 for ethanol, methanol, ethyl acetate and ascorbic acid standard respectively.

. H_2O_2 scavenging activity

The scavenging of H_2O_2 by the leaf extracts of *T. acuminata*are presented in Figure 3. Among 3 different leaf extracts of *T. Acuminata*, the ethyl acetate extract was capable of scavenging H_2O_2 significantly in a concentration dependent manner and IC_{50} was found to be 159, 108, 54, 48 for ethanol, methanol, ethyl acetate and ascorbic acid standard . respectively.

Antibacterial activity

The leaf extracts of *T. acuminata* were tested for its antibacterial activity at a certain concentration 100µg/well against 6 pathogenic organisms (Figure 4). Methanol extract shoed zone of inhibition against all organisms except *K. pneumonia* and Ethyl acetate produced inhibition zones in all organisms except *B. subtilis*. The maximum inhibitory zone was produced by Methanol extract against *P. aeruginosa*. The ethanol extract produced least response with zone of inhibitions against only two organisms viz. *E. coli* and *P. aeruginosa* (Figure 4).

Brine shrimp lethality test

As a result of strong antimicrobial and antioxidant activities, ethyl acetate extract of *T. acuminata* was selected for further investigation on brine shrimp lethality test and anticancer activity assay. After enumerating the number of shrimps surviving after 24 h, the percentage inhibition was evaluated. The lethality concentration (LC_{50}) was found to be 85µg (Figure 5). The 100% inhibition of cell line was obtained with 100 µg/ml concentration of the extract. So this study proved that ethyl acetate extract of *T. acuminata* could exhibit a lethal activity against brine shrimp.

Antiproliferative activity

The antiproliferative activities of ethyl acetate extract of *T. acuminata* on the growth of cell line *in vitro* (Figure 5). At the concentration of 50µg/mL, the ethyl acetate extract showed 50% antiproliferative activity against the cell line with the corresponding inhibitory activities of 73.33 under the experimental conditions.

DISCUSSION

The results revealed that the ethyl acetate is regarded as the most effective solvent for extracting phenolics from *T. acuminata*. The total phenolic and flavanoid content of the ethyl acetate was higher than those of the methanol and ethanol extract. This could be explained by the possible formation of complexes of certain part of the phenolic compound with other components, which are more

extractable in ethyl acetate than those of other extracts [20, 21]. Oxidation processes are intrinsic in the energy management of all living organisms and are therefore, kept under strict control by several cellular mechanisms [22]. However, the aberrant production and unbalanced mechanisms of antioxidant protection leads to several human diseases and conditions such as cancer, diabetes, inflammatory disorders, as well as aging processes etc. [23, 24]. Antioxidants are considered as possible protective agents reducing oxidative damage to the human body [25]. Antioxidants are naturally abundant in fruits and are able to neutralize free radicals donating an electron and converting them to harmless molecules [26].

The present study is a step towards the exploration of natural antioxidants from leaf extracts of *T. acuminata* employing free radical scavenging assays in addition to anti-proliferative and antimicrobial activities. The results of our study reveal that there is a strong coincidence between antioxidant activity and phenolic content. Several studies on the total phenolic content had been published over the years demonstrating its importance in the medicinal field [27-29]. It is clear that the antioxidant activity of *T. acuminata* extracts in DPPH assay increased proportionally to the polyphenol content and same trend was observed in earlier reports where increased antioxidant activities showed linear relationships between DPPH values and total polyphenols [30, 31].

Hydrogen peroxide is an oxidant that is being continuously generated in living tissues as a result of several metabolic processes. The detoxification of H_2O_2 is vital for preventing it from reacting in damaging Fenton-type reactions, which generate extremely reactive oxygen species including hydroxyl free radical [32]. As shown from this study, *T. acuminata* extracts have an effective free radical scavenging activity for H_2O_2 in a concentration dependent manner and results reveal that these extracts have a significant scavenging character in accordance with the ascorbic acid standard (Figure 3). Similar results have shown that scavenging of H_2O_2 by extracts may be attributed to their phenolics, which can donate electrons to H_2O_2 and neutralize it to water [33, 34].

There has been a 22% increase in cancer incidence and mortality, with over 10 million new cases and over 6 million deaths worldwide in the year 2000 and cases could further increase by 50% to 15 million new cases in the year 2020 [35]. The use of medicinal plant and fruit extracts for cancer therapy is rapidly evolving as they are affordable, with limited or no side effects. The active components present in such extracts have been shown to efficiently inhibit the processing of multistage carcinogenesis in a synergistic manner. The identification and characterization of components with potential anti-cancer activity derived from herbal or medicinal plant extracts has been gaining attention. Earlier reports revealed that the antioxidant activity prevents the development of cancers [36-39]. So in this context, we have also examined the antiproliferative ability of *T. acuminata* extracts using HEp2 cancer cell line. We found that the proliferation was inhibited in a concentration dependent manner after the exposure to the plant extract. The cytotoxicity was higher in ethyl acetate extract than the other solvent extracts in the cell line tested. Although, the activity is low in comparison to the standard drug, this may be due to the crude nature of the extracts, which can be further enhanced by the purification. It can be inferred that ethyl acetate extracts of *T. acuminata* might be useful as an antiproliferative agent due to the presence of potent bioactive principles [40].

Furthermore, medicinal herbs had been used in ayuverdic traditional medicine for their effectiveness against a wide range of diseases due to the advantage of diverse secondary metabolites such as phenolic compounds including flavonoids, alkaloids and tannins [41-43].

Therefore, we also examined the antimicrobial activity of *T. acuminata* extracts against a panel of six pathogenic bacteria. Methanol and ethyl acetate extracts exhibited higher degrees of antimicrobial activity than the other extract.

Brine shrimp lethality is a general bioassay, which is indicative of cytotoxicity of an extract or compound [44]. This is attested to by the large difference in their LD_{50} values. However, all the extracts can still be considered safe due to their relatively high LD_{50}. Anderson *et al* [45] stated that extracts which showed LD_{50} higher than 100µg/ml in the brine shrimp lethality test can be considered inactive and so safe for consumption.

GC-MS analysis of ethyl acetate extract of *T. acuminata* revealed the presence of three important compounds (Table 3): Neophytadiene, Phytol and Testosterone capionate. Neophytadiene belongs to a key flavour compound and represents “heart of Tobacco”. Neophytadiene presence in *T. acuminata* (37.5%) is higher than in Virginia (25.0%) and Burley (33.4%) [46]. Tetramethyl-2-hexdecene-1-ol is an important class of compound known as Phytol. Phytol treatment deceases the autoimmune response and to ameliorate both acute and chronic phases of arthritis [47]. Testosterone cypionate is useful in improving fertility of male [48] and also decreases ST segment depression in post exercise electrocardiogram [49].

Based on the FT-IR spectrum (Figure 1), it may be concluded that band 1 contains a compound of polyphenolic nature. The presence of lactone rings indicates the presence of flavonoids or coumarins. Band 2 was a single spot indicative of one compound. The IR spectrum of band 2 showed a broad peak at 3834.2 cm-1 indicative of a hydroxyl group (-OH), a sharp peak at 1650.9 cm-1 indicative of the carbonyl group. Therefore, this band must possess compounds that are having a hydroxyl and carbonyl group [50]. Thus, the results and observations presented clearly demonstrate the presence of compounds such as alkaloids and polyphenols in the leaves of *T. acuminata*.

We conclude that this study proved the presence of potential bio-active compounds with various activities in the extracts of *T. acuminata*. Further studies on this species will help to identify a most potent plant based drug for treating important diseases.

CONFLICT OF INTERESTS

Declared None

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