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RESEARCH ARTICLE

Phytochemical Screening and Potential of Antimicrobial and Anti-inflammatory Activity of Organic Extract Derived from *Tragia plukenetii* Radcl. Sm

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Abstract

Plants and other natural sources are the only remedial agents to cure the health illness and health maintenance up to the development of modern technology. Later, the Technological advancement creates a lot to protect ourselves using synthetic drugs from the various human health complications with some side effects. In addition, the evolution of new pathogenic bacterial strains has developed a resistance against regularly used antibiotics is a prime anxiety for global health community. Such side effects are needed to be nullified, hence, it is necessary to discover new biologically active drugs to treat and inhibit the pathogenic organisms. Therefore, the usage of medicinal plants is the better remedial source to overcome these problems. With this concern, we have used the medicinal plant *Tragia plukenetii* Radcl.Sandm in this study and this plant extract was collected using Water, Petroleum ether, Ethanol and Methanol. As a result, Carbohydrates, Proteins, Amino acids, Flavonoids were found more while; Terpenoids were absent in all the extract except Water. Moreover, Alkaloids and Tannins were present in all the solvent extract. *In vitro* antibacterial potential against different human bacterial pathogens, haemolysis and anti-inflammatory properties were tested for all the solvent extracts. The highest antibacterial and anti-inflammatory activity were found in the Water extracts against all the tested organisms when compared to other solvents such as Methanol, Ethanol, and Petroleum Ether extracts. This finding supports the plant *T. plukenetii* could be potentially used as a nutraceutical for antibacterial and anti-inflammatory properties.

Keywords: *Tragia plukenetii,* Phytochemical screening, Heamolysis, Antibacterial and Anti-inflammatory activity.

Introduction

In India, around 20,000 medicinal plants have been identified recently, however more than 500 traditional communities were used about 800 plant species for curing different diseases (Kalaivani *et al.* 2012). The value of medicinal plants to mankind is very well-proven. It is estimated that, about 70 to 80% of the people worldwide rely chiefly on the traditional health care system and primarily on herbal medicines (Sathiyaraj *et al*. 2015). Ayurveda and Unani system of medicines were regularly employed from a large number of Indian medicinal plants are reservoir of novel source of medication used as a potential antibiotic agent for different diseases (Sahaya Sathish *et al.* 2016), because their chemical agents with therapeutic properties (Sandigawad 2010). Plant extracts has plenty of array of phytochemicals, which has antimicrobial and anti-inflammatory properties with great biological significance. Therefore, screening and testing the efficacy of plant extracts are embraced to investigate their antibacterial and anti-inflammatory activity (Bhardwaj and Laura 2009; Gopalakrishnan *et al.* 2013).

The *Tragia plukenetii* R. Smith is called as Senthatti in Tamil and Duradagondi in Telugu and it is widely distributed in India, Somalia, Ethiopia, Kenya and Tanzania. The leaf of this plant is palmately 3-partite, pinnatifid. Traditionally, this plant has been used to treat sore throat (Yoganarasimhan 2000). The medicinal properties of Ethanolic extract of *T. plukenetii* leaf are due to the presence of phytoconstituents

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like alkaloids, Flavonoids, Glycosides, Sterols, Saponins and Tannins (Reddy *et al.* 2017). Flavonoids are widely distributed in *T. plukenetii* in the form of glycosides along with 16 different compounds. In addition, the steroid (TP-I) and isoquinoline (TP-II) are extracted from *T. plukenetii* (Stanley *et al.* (2012).

Medicinal and aromatic plants are used in a large scale for phytomedicine against different drug-resistant bacteria, which are considered as one of the most important reasons for the success of treatment in infectious diseases. Medicinal plants are the primary sources of new medicines and may represent an alternative to the usual drugs (Al-Mariri and Mazen Safi 2014). Some of the *Staphylococcus* sp. and *Streptococcus* sp. involved in the respiratory and skin infections, along with *Pseudomonas* sp. and the members of the Enterobacteriaceae causes gastrointestinal, urogenital diseases and wound contamination, they are resistant to virtually all of the older antibiotics. Clinical isolates of *Staphylococcus aureus*, the leading cause of nosocomial infections, are increasingly resistant to an array of antimicrobial agents like Penicillin, Gentamicin, Tobramycin, Amikacin, Ciprofloxacin, Clindamycin, Erythromycin, Chloramphenicol, Trimethoprim-sulfamethoxazole and Vancomycin (Elisha *et al.* 2017).

Inflammation is a suitable physiological process for the body to preserve homeostasis when tissue under damage of stress (Umapathy *et al*. 2010, Abdelmagid *et al.* 2015). Damage of living tissues caused by means of pathogenic agents (bacteria, virus, fungus), physical agents such as heat, radiation and corrosive chemicals are the active cause for the inflammation, sometimes defective immune also responsible for the inflammation (Barnes 2009, Hernandez *et al*. 2011). Hence it is very important to focus on the elimination of toxic agents and to confiscate injured tissue materials for the repairing damaged tissue.

Herein we demonstrated, extraction of crude metabolites form *Tragia plukenetii* Radcl.-Sm, phyto chemical screening, antibacterial and anti-inflammatory properties of the extract against Gram-positive and Gram-negative organisms. However, comprehensive scientific investigations are required to access the usefulness of the entire plant to treat the Bacterial diseases. The root of the *T. plukenetii* Radcl.-Sm., is effectively used to cure male impotence in Somalia (Stanley *et al*. 2012). There is no report regarding biological activities of *T. plukenetii* extract. Hence, this study aims to investigate the Phytochemical screening, antibacterial activity and anti-inflammatory.

Materials And Methods

A. Plant collection and identification

Tragia plukenetii Radcl.-Sm is an important medicinal herb used in the present study and the mature, healthy and disease-free plants were collected from Sivakasi, Alagiyanallur and in and around the area of Virudhunagar District of Tamil Nadu and taxonomically identified and authenticated by the taxonomist Dr.A.Sarvalingam, Assistant Professor, Department of Botany, Sri Kaliswari College, Sivakasi (Autonomous). A voucher specimen was deposited in SKC Herbarium, The Department of Botany Sri Kaliswari College, Sivakasi, Tamil Nadu, India.

B. Plant description

Twining climber, up to 1.5 m; leaves simple, leaf blade commonly deeply trilobate or tripartite, occasionally with some leaves lobate, rarely quinquelobate, sparingly hispid or setose dark green above, paler beneath, the midrib and main nerves often reddish. Inflorescence: Raceme flowers small, white to creame colour, tapels lanceshaped and toothed margins; Fruits trilobate, 4–5 mm. long, 7–8 mm. diameter, smooth, sparingly to evenly adpressed - setose; Seeds 2.5–3 mm. diameter, orange- to reddish-brown, mottled buff or grayish (Figure 1)

Flower and fruiting: September – February

Distribution: India - Maharashtra: Ahemdnagar, Pune, Satara, Karnataka: Chikmagalur, Coorg and All districts in Tamil Nadu.

Useful part: Whole plants

Ethnomedicinal Uses: Blood purifier in veneral diseases. Roots are diaphoretic, alternative, diuretic and blood purifier. The roots are used to cure male impotence.

C. Preparation of Plant Extract

The 50gm of shade dried powder of the whole plant of *Tragia plukenetii* Radcl.-Sm was packed well in Soxhlet apparatus and the plant extract was prepared using different solvent systems such as Petroleum Ether, Ethanol, Methanol, and Water by providing continuous heat for about 24hrs (Ferrazzano *et al.* 2013). The plant extract of each solvent

Figure 1: Habit of the plant *Tragia plukenetii*

system was concentrated under vacuum distillation and then weighed individually. The marc was then subjected into extraction as above with methanol. The dried methanol extract was suspended in water and fractionated with ethyl acetate. The soluble portion of ethyl acetate was separated, concentrated under vacuum distillation and then weighed (Krishnaiah *et al.* 2009). The prepared extracts were used for phytochemical investigation by standard methods (Gowri and Vasantha 2010, Takaidza *et al.* 2018).

D. Phytochemical analysis

Phytochemical examinations were carried out for the plant extract collected from all solvent systems all the exacts (Petroleum Ether, Ethanol, Methanol, and Water) as per the standard methods to find out the presence of pharmacologically active constituents such as Phenols, Alkaloids, Saponins, Flavonoids, Terpenoids, Cardiac Glycosides, Steroids, Tannins and Carbohydrates (Madike *et al.* 2017).

Test for Alkaloids

About 50 mg of *T. plukenetii* extract was added with 2-4 mL of diluted Hydrochloric acid and few drops of Wagner's reagent were added at the side of the test tube. The reactants were mixed well, the appearance of reddish-brown precipitate indicates the presence of alkaloids (Madike *et al.* 2017).

Test for Flavonoids

Few drops of diluted Sodium hydroxide solution was added into the *T. plukenetii* extract (0.5 ml) to give intense yellow colour which disappears after addition of diluted hydrochloride acid showed the presence of Flavonoids (Dhakad 2017).

Test of Carbohydrates Molisch test

To 2–3 ml of the aqueous extract of was *T. plukenetii* extract added two drops of alpha-naphthol solution, shaken well and added conc. H_2SO_4 from the sides of the test tube. Violet ring was formed (Boxi *et al*. 2000).

Benedict test

The plant extract was treated with Benedict's reagent and heated gently. The Orange-red precipitate formation was observed (Madike *et al.* 2017).

Test for proteins and Amino acids

Ninhydrin test

To the *T. plukenetii* extract, 0.25% Ninhydrin reagent was added and boiled for a few minutes. Formation of blue colour indicates the presence of Amino acid (Harborne 1973).

Biuret test

The plant extracts were treated with a few drops of 5% NaOH & 1% $CuSO_4$. Formation of yellow colour indicates the presence of proteins (Harborne 1973).

Test for tannins

1g of *T. plukenetii* extract dissolved in water in a test tube, it was diluted with Chloroform and 1 mL of Acetic anhydride was added. Finally, Sulphuric acid (1 mL) was added carefully to the side of the test tube to the solution. The appearance of green colour was formed which confirmed the presence of Tannins (Hossain *et al*. 2013).

Test for Terpenoids

0.5 mg of the plant extract was added in a 2-5 mL of Chloroform followed by concentrated Sulphuric acid. Formation of the reddish-brown ring at the interface indicated the presence of Terpenoids (Hossain *et al*. 2013).

E. Antibacterial Activity

Test organism

Antibacterial activity was performed against five different Human bacterial pathogens such as *Micrococcus*, *Strptomycetes*, *Salmonella typhii*, *E.coli*, *S.marseaceae.* 12 hrs old bacterial culture was inoculated into the nutrient broth and incubated on a rotary shaker at 37 ± 2° C at 100 rpm.

Different concentrations of plant extracts were tested for its antimicrobial potential by Agar well diffusion method (Varatharaju *et al*. 2020). Microbial suspension was evenly spread on the sterile agar medium using sterile cotton swap, 6mm diameter of wells were made in the agar medium using sterile cork borer and the wells were loaded with 40 µL of *T. plukenetii* extract. This method was applied to all the solvent extracts along with control. The inoculated tests and control plates were kept at room temperature for few minutes and incubated at 37° C for 24 to 48 h. The zone of inhibition (ZOI) around the wells was measured and the area of inhibition zone was calculated (Suresh *et al.* 2021).

In-vitro Anti- Inflammatory

In-vitro study of Anti-inflammatory property of the *T. plukenetii* extract was tested by membrane stabilization method using Sheep red blood cell (SRBC) (Nainwani *et al*. 2014). Sheep blood was collected from healthy Sheep for two weeks before the experiment and it was mixed with an equal volume of Alsever's solution (2% dextrose, 0.8% Disodium citrate, 0.5% Citric acidic acid and 0.42% of NaCl) and it was centrifuged at 3000 rpm. The packed cells were washed with Isosaline and 10% suspension was made from it. The Hydro-alcoholic extract was prepared in the concentration of 100, 200, 400, 800 µg/mL using distilled water and 1mL of Phosphate buffer, 2M Hypo saline and 0.5M of SRBC were added to each concentration. It was incubated at 37° C for 30 minutes and centrifuged at 3000 rpm for 20 minutes. The Haemoglobin content in the supernatant was estimated using Spectrophotometer at 560 nm. Diclofenac (100, 200, 400, 800 µg/mL) was used as a standard and control was prepared as the test solution was prepared without the

T. plukenetii extract. The percentage of SRBC membrane stabilization or protection was confirmed based on the range of OD value. The percentage inhibition of protein denaturation was calculated by using the following formula (Banerjee *et al.,* 2014):

% Inhibition = 100 × ([Vt/Vc] –1).

Where, Vt = absorbance of test sample, Vc = absorbance of control.

Results and Discussion

From the ancient Siddhar period to present modern medicine, people are depended and used natural products to fight against different pathogens to protect the human beings and their pet animals or to maintain the healthy body. Plants are the major and important source of medicinally valuable novel compounds; hence the Plants are generally called as nature's gifts. Screening of biochemical constituents available in the plants is the basic foundation of the research to find out the novel chemo-preventive representative molecules and their biological importance to explore the remedial applications to cure the disease (Khan *et al.* 2018). Many researchers found out the accumulation of Phenols, Flavones, Quinones, Flavonols, Terpenoids, Coumarins, Tannins, Essential oils, Polyamines, Glycosides, Alkaloids and many more bioactive compounds present in the different medicinal plants, which has high impact on antimicrobial, anti inflammatory potential (Stavri *et al.* 2007). Even though, these groups of phyto-chemicals were reported for their antimicrobial properties, they are not appreciated as therapeutic molecules by the medical communal for few reasons (Gibbons *et al.* 2004).

The most effective solvent extraction for Polyphenolic compounds from plants is Hot percolation technique than other extraction methods (Azwanida *et al.* 2015). The dried leaves of *Moringa oleifera* has given a maximum amounts of total phenolics compound (13.23 g) which was obtained from 70% ethanol and also total flavonoids (6.20 g) (Vongsak *et al.* 2013). In this study, the extract yield percentage of *T. plukenetii* is shown in (Table 1).

The Ethanol (9.58%) and Methanol (8.98%) extracts showed higher recovery percentage when compared with other solvents. On the other hand, Petroleum ether extract (4.47%) showed lower percentage among the other extracts.

Qualitative phytochemical analysis

Primary metabolites such as Carbohydrates, Proteins and Amino acids were evaluated from the solvent extract of *T. plukenetii* and the secondary metabolites such as Alkaloids, Tannins and Flavonoids were found at a different level in the *T. plukenetii* extract (Table 2).

The result of *T. plukenetii* extract showed Proteins, Alkaloids, Flavonoids and Tannins were found more while; Carbohydrates, Amino acids, Terpenoids were absent in the Methanol extract. Water extract of *T. plukenetii* exhibited the presence of all the molecules which were absent in the other solvent systems.

Table 1: The percentage yield of *T. plukenetii* extracts from different solvent system

S. No.	Solvent systems	Whole Plant (%)
1	Petroleum Ether	4.47
2	Ethanol	9.58
3	Methanol	8.98
4	Water	5.64

Carbohydrates, Amino acids and Proteins constitute a major class of naturally occurring organic compounds essential for plant and animal life and also provide raw materials for many pharmacological investigations. The phenolic compounds are one of the most abundant and most ubiquitous groups of plant metabolites (Singh *et al.* 2007) having different biological functions such as anti-microbial, anti-ageing, anti-carcinogen, anti-inflammation, anti-atherosclerosis, cardiovascular protection and also involved in the improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation (Han *et al.* 2007). Several studies have described that, the antioxidant properties of different medicinal plants are rich in Phenolic compounds (Krings and Berger 2001). Proteins are structural molecules for most of the pathogenic microorganisms, the plant derived Tannins are played major destructive function in precipitating such proteins by which the development of pathogenic microorganisms are effectively suppressed (Madike *et al.* 2017). Tannins are also used for the treatment of non-specific diarrhoea, mouth inflammation, throat infection and for skin allergy (Dhakad 2017). Like Tannins, Flavonoids are also has positive response to treat against microbial infection (Vongsak *et al.* 2013).

Antibacterial activity of T. plukenetii extract

The results of the present study revealed that, the antibacterial efficacy of Water, Ethanol, Methanol and

Table 2: Phytochemical analysis of *T. plukenetii* extract

S.No	*Phyto-chemical constituents*	*Water*	*Methanol*	*Ethanol*	*Petroleum Ether*
1	Carbohydrate	+	-	-	-
2	Aminoacid	+	-	-	-
3	Protein	+	+	+	+
4	Alkaloids	+	+	+	+
5	Flavonoids	+	+	+	-
6	Terpenoids	+	-	-	-
7	Tannins	+	+	+	+

(+): Presence of chemical compound, (-): Absence of chemical compound

Petroleum ether extracts showed varied level of inhibition which may be attributed to the presence of Tannin and Saponins at different level. The Phenolic compounds of the plant has the antimicrobial potential, the antibacterial activity of plant extract which agrees with the findings of Okwu and Josaiah (2006). Both Benzene and Petroleum ether extracts were found to be ineffective of bacterial inhibition, due to the presence of fewer numbers of active compounds, Saponins, Steroids and Alkaloids. The present study revealed that, the tested plant *T. plukenetii* extract posses potential bacterial inhibition against gram-positive organisms such as *Micrococcus, Streptomyces* and gram-negative organisms such as *Pseduomonas aeruginesa*, *Salmonella typhi*, *E.coil*, and *Serattia maresansa* (Figure 2).

Methanol extract of *T. plukenetii* has effective against both Gram-positive and Gram-negative bacteria. These findings exhibited some differences with extracts although the Water, Petroleum ether and Ethanol extract have a considerable inhibitory effect. The growth of *S.marsececae* was significantly arrested by all the solvent extract of *T. plukenetii* compared to other organisms and Petroleum ether extract developed a maximum inhibition than other solvent extracts. In addition, *Micrococcus* was potentially inhibited by water extract of *T. plukenetii* than other solvent extracts (Table 3).

The contrast between the results may be caused by the difference in extraction methods from *T. plukenetii,* which showed positive and negative antibacterial against all bacterial strains. Plant-based antimicrobial compounds have enormous therapeutic potential as they can serve the purpose without any side effects that are often associated with synthetic antimicrobials. Results of the present study are found directly correlated with the observations of previous findings.

E. coli strains are occasionally responsible for food contamination (Vogt and Dippold, 2005), hence the results suggest that *T. plukenetii* extract can be effectively used to protect against *E. coli* related infections. *Staphylo coccus* cause Staph infections (Podschun and Ullman 1998) which was considerably inhibited by *T. plukenetii* extract, so the result suggest that, the plant extract may be used to cure these infectious diseases.

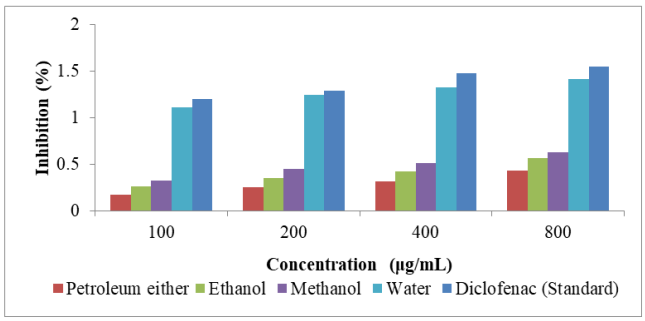

Figure 2: In vitro anti-Inflammatory activity of *T. plukenetii*

Table 3: Antibacterial activity of *Tragia plukenetii* extract

Name of the Organism	*Water*	*Methanol*	*Ethanol*	*Petroleum ether*
	Zone of Inhibition (mm)			
Micrococcus	15	12	10	---
Streptomyces	14	12	10	---
E.coli	---	8	---	7
S.marsececae	7	7	7	13
S.typhi	---	6	5	5

Flavonoids from plant source have been referred to as nature's biological response modifiers by their inherent ability to allergen, Bacteria, Virus and carcinogens. The experimental evidence proved that, the Flavonoids has anti-allergic, anti-inflammatory, antimicrobial and anticancer activity (Cushnie and Lamb 2005). Moreover, Tannins are generally known to possess antimicrobial and antioxidant activities (Rievere *et al.,* 2009). Since the Flavonoids and Tannins present in the plant extracts of *T. plukenetii,* it may contribute to the various biological activity such as antimicrobial, Heamolysis and anti-inflammatory activities.

In vitro Anti-Inflammatory activity

Inflammation is one of the root causes for the release of lysosomal enzymes from the damaged tissues which leads to different types of disorders like acute or chronic inflammation (Rajurkar *et al*. 2009). The test towards anti-inflammatory activities of *T. plukenetii* extracts are provided higher inhibition in water extracts (1.41%) which is comparable to standard results. The methanol extract of *T. plukenetii* produced next level of significant result (0.63.00%) at 800 µg/mL concentration. The Petroleum ether and Ethanol extract of *T. plukenetii* shows moderate inhibition (0.56%) (Figure 3).

The Ethanolic extract of *A. indicum* has the significant suppression of inflammation indicated the long duration of anti-inflammatory action of the plant extract (Kumar *et al*. 2013). Erythrocytes are affected by the inflammatory mediators whose formation is triggered by injuries and foreign particles. Compound with membrane-stabilizing properties offers significant protection of the cell membrane against from harmful substances by releasing phospholipases, which control the formation of

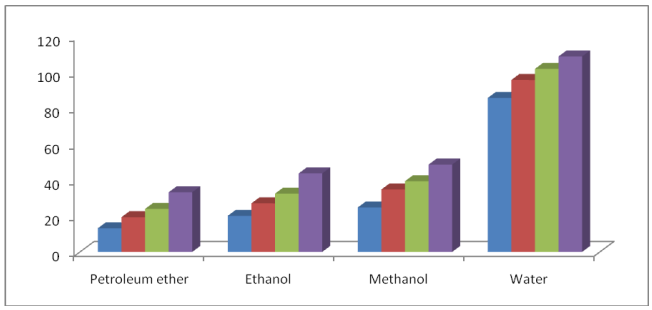

Figure 3: In vitro analysis of Haemolysis activity of *T. plukenetii*

inflammatory mediators (Varatharaju *et al*. 2013). When, HRBCs are exposed to hypotonic medium which causes membrane lysis and oxidation of haemoglobin. The haemolytic effect of the hypotonic solution is related to excessive accumulation of fluid within the cell resulting in the rupturing of its membrane causing secondary damage through free radical-induced lipid peroxidation (Kumar *et al*. 2013). As indicated in the results, the extracts of *T. plukenetii* exhibited excellent antioxidant property, in addition to its anti-inflammatory property. These results suggest that, the plant could serve as an excellent anti-inflammatory agent analogously reducing the oxidative stress.

In-vitro Analysis of Haemolysis activity of T. plukenetii extract

The biologically active compounds may not be useful in pharmacological preparations because they may possess hemolytic effect. Therefore, Hemolytic assay was performed the functional property of *T. plukenetii* extract. Besides, the assay results may also reveal some information about the mechanism of cytotoxicity. *In vitro* hemolytic activity on erythrocytes using various concentrations of extracts obtained from *T. plukenetii* was analyzed. The total hemolysis was obtained using Triton X-100 (0.1%) and as a result 0% hemolysis was obtained with buffer (Table 4).

The hemolysis induced by extracts in red blood cells was concentration-dependent but the results of all extract showed the lower hemolytic effect on human red blood cell in all the tested concentrations of an extract from *T. plukenetii*. The erythrocyte model has been widely used as it presents a direct indication of toxicity of injectable formulations as well as general evidence of membrane toxicity. Another advantage of erythrocytes model is that, the blood is readily available and easy to isolate the cell from the blood. Moreover, its membrane has similarities with other cell membranes (Robertis 1995). Erythrocytes have been used as a model system by several researchers to understand the interaction profile of drugs with membranes (Costa Lotufo *et al.* 2005) which resulted from lysis of membrane lipid bilayer. This hemolysis relates to concentration and potency of the extract. Furthermore, the hemolytic activity of each extract is related to their chemical composition. Our results showed more ability of erythrocyte to hydrolysis in the presence of extracts from *T. plukenetii*. It was reported that the methanol extract from the *T. plukenetii*, antibacterial, antimycotic activities and are used in folk medicine to evacuate coagulated blood and trait hematomas.

Table 4: In vitro analysis of Heamolysis activity of *T. plukenetii* extract

S.No	*Concentration (mg/µL)*	*Petroleum Ether*	*Ethanol*	*Methanol*	*Water*
1	100	13.1355	20.0896	24.7256	85.7672
2	200	19.3169	27.0437	34.7705	95.8120
3	400	23.9530	32.4524	39.4065	101.9935
4	800	33.2251	43.2699	48.6787	108.9461

Conclusion

Form the research conducted, it is concluded that *T. plukenetii* can exhibit antibacterial activity against different bacterial pathogens and anti-inflammatory activity because of the presence of phytochemical such as Alkaloids, Terpenoids and Flavonoids. There are several factors which can affect their antimicrobial potential such as resistance of tested bacterial strains, variation in preparation condition and low concentration of bioactive phytochemical present in the *T. plukenetii* extract.

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