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EVALUATION OF IN-VITRO ANTI-ULCER ACTIVITY OF BARK EXTRACT OF *TRACHYSPERMUM AMMI* (AJWAIN)

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Abstract

The current study's findings show that many phytochemicals, including polyphenolic components such alkaloids, flavonoids, tannins, steroids, and phenols, resins, and carbohydrates, are present in the ethanolic extract of Trachysprmum ammi bark. Ethnobotanical and traditional uses of natural substances, particularly those derived from plants, have drawn a lot of attention recently as they have been scientifically proven to be safe for human use and to be beneficial. When looking for novel compounds to treat a variety of illnesses, this traditional method works the best. A thorough review of the literature on Trachyspermum ammi revealed that traditional healers and members of different ethnic groups frequently use it as a therapy for a variety of illnesses. Based on the results, we can conclude that the ethanolic extract of *Trachyspermumammi* bark can be attributed to Anti-oxidant activity and Anti-ulcer activity. However, theisolation of active constituents from the ethanolic extract of *Trachyspermum ammi* bark species and its action responsible for its Anti-oxidant activity and Anti-ulcer activity effectcan be useful for the treatment of the same in future.

Keywords: Trachyspermum ammi, Anti-oxidant activity and Anti-ulcer activity.

Introduction:

Peptic ulcer is a broad term which includes ulcers of digestive tract in the stomach or the duodenum. Earlier it was believed that, one can developed this type of ulcers due to stress and spicy food. However, recent research has shown that these are just the aggravating factors. Peptic ulcer are the areas of degeneration and necrosis of gastrointestinal mucosa exposed to acid-peptic secretions. Though they can occur at any level of alimentary tract that is exposed to hydrochloric acid and pepsin, they occur most commonly (98- 99%) in either the duodenum or the stomach in the ratio of 4:1 each of two main types may be acute or chronic. The causes of peptic ulcers include infection caused by the bacteria *H. pylori*, smoking or reaction to certain medicines like non-steroidal anti-inflammatory drugs (NASID's).

These ulcers occur following severe stress. The causes are as follows:

1. Psychological stress
2. Physiological stress as in the following:

- Shock
- Severe trauma
- Septicaemia

Peptic ulcers are more frequent in middle aged adults. The peak incidence for duodenal ulcer 5th decade, while for gastric ulcer it is decade later (6th decade). Duodenal as well as gastric ulcers are more common in males than in females.Duodenal ulcer is also four times more common than gastric ulcer; the overall incidence of gastroduodenal ulcers being approximately 10% of the male population. [1]

Statistics from all sources indicate 10% or more of adult population are affected with a high prevalence of *H. pylori* infection, morbidity and mortality of peptic ulcer disease (PUD) continue to be increased. 10% or more of adult population are affected within their life time. Peptic ulcer occurs more often in individuals from 20to 60 years of age. [2] In some populations, the prevalence has been documented as being as high as 50- 66% and it is especially common in North America. The incidence of aphthous ulcers has been found to be lower in smokers than in non- smokers. [3] Patients, who underwent surgery for peptic ulcer disease (PUD) in Sub- Saharan Africa, indicated that 86% suffered from duodenal ulcers while the rest 14% had gastric ulcer. (Major complications like perforation (35%), bleeding (7%), obstruction (30%), and chronic case (28%) were indicated for surgery, and the overall fatality rate was found to be 5.7%. [4]

Peptic ulcer disease (PUD) develops when the protective mechanisms of the gastrointestinal mucosa, such as mucus and bicarbonate secretion, are overwhelmed by the damaging effects of gastric acid and pepsin. Peptic ulcers occur mainly in the stomach [gastric ulcer (GU)] or proximal duodenum [duodenal ulcer (DU)]. The usage of medicinal plants in healing numerous diseases is as old as human beings, and well-known as phytotherapy. Moreover, in the past few years, there has been a rising interest in alternative therapies and the usage of herbal products, in particular, those produced from medicinal plants. [5,6] Also, due to appearance of various side effects by usage of conventional drugs for numerous diseases, medicinal plants are considered the major reservoir of potentially new drugs. Plant extracts and their crude are the most significant sources of new drugs, and have been shown to cause promising results in the treatment of gastric ulcer as well. [7] *Trachyspermum Ammi* possesses biological effects such as antiviral, anti- inflammatory, antifungal, antipyretic, anti-filarial, analgesic, anti- nociceptive and antioxidant activity have been confirmed. Also, there are traditional claims that *Trachyspermum Ammi* is used as gastroprotective. Literature review revealed that there is no current research reported for its gastroprotective activity. So, the present study is planned to explore antiulcer potential of *Trachyspermum Ammi* bark extract to authenticate and reconfirm the traditional claims.

Trachyspermum Ammi:

Fig no. 1: *Trachyspermum Ammi* (fruit) & (leaves)

Medicinal plants always played an important role in the health development of mankind. *Trachyspermum ammi* is one of the oldest spice plants which, due to its economic importance and significant pharmaceutical industry applications.*Trachyspermum ammi*, belonging to *Apiaceae* family comprising 270 genera and species. *Trachyspermum ammi* is native of Egypt and grows widely around Mediterranean Sea and in Southwest Asia. It is cultivated in India, Iran, Afghanistan, Pakistan and Iraq. The most commonly used part of this plant is the seeds or fruit. Essential oils possess various biological activities such as antioxidant, antibacterial, anti-mutagenic and antimicrobial, seeds have higher energy value that is 314.55%. It is rich source of carbohydrates 47.54%. Protein, moisture, ash and fiber contents are present in the rangeof 4.30%- 20.23%; fat contents were in the range of 4.83% these leaves are used as green vegetable for salad. [8]

1. Digestion: *Ajwain* seeds are also known as a digestive aid, and combines well with fennel to relieve gas and bloating.
2. Colds and Flu: *Ajwain* seeds contain about 50% thymol, a well-known andantibacterial essential oil, and along with thyme can be used to enhance theimmune system to ward off colds and flu and other viral infections.
3. For relieving flatulence, dyspepsia and spasmodic disorders; a teaspoonful of *Ajwain* seeds with a little amount of rock-salt, mixed with water taken internally. [9,10]

Etiology:

The etiology of peptic ulcer is generallyaccepted that it results from an imbalance between aggressive factors and the maintenance of mucosal integrity through the endogenous defense mechanism. [11] Acidity is a common gastrointestinal problem which is attributed to a functional disorder that can result due to a variety of reasons. Antacids act by neutralizing gastricacid and thereby reduce the gastric pH. [12] Excessive secretion of gastric acid or stomachacid (i.e., HCl), inflames the stomach lining and produces ulceration [13]. Antacids act byneutralizing gastric acid and thereby reduce the gastric pH. The regain balance is maintained for the use of therapeutic agents differently for the use of gastric acid secretion inhibition or by increasing the mucosal production to boost the mucosal defense mechanism by stabilizing the surface epithelial cells or inhibition of prostaglandin synthesis. The acid-neutralizing

capacity (ANC) of an antacid is the amount of acid that it can neutralize. [14]

NSAIDs disrupt mucus phospholipids and lead to the uncoupling of mitochondrial oxidative phosphorylation, thus initiating mucosal damage. When exposed to acidic gastric juice (pH 2), NSAIDs become protonated and cross lipid membranes to enter epithelial cells (pH 7.4), where they ionize and release H+. In that form, NSAIDs cannot cross the lipid membrane, and are trapped in epithelial cells, leading to the uncoupling of oxidative phosphorylation, decreased mitochondrial energy production, increased cellular permeability, and reduced cellular integrity. Patients who have a history of peptic ulcers or hemorrhage, are over the age of 65, also use steroids or anticoagulants, and take high doses or combinations of NSAIDs are at the highest risk for acquiring NSAID-induced ulcers. [15]

MATERIALS AND METHODS

A. List of chemicals:

Chemicals Manufacturer

1, 1 diphenyl 2- picryl hydrazyl	SRL Manufacturers
Al (OH)3, Mg (OH)2	SRL Manufacturers
Phenophthalein	SRL Manufacturers
Tri's buffer	SRL Manufacturers
Magnesium chloride	SRL Manufacturers
Thiourea	SRL Manufacturers
Potassium phosphate buffer	SRL Manufacturers
Ammonium molybdate	SRL Manufacturers
Perchloric acid	Loba chemicals

B. List of instruments:

Instruments	Manufacturer
Weighing balance	Shimadzu AUY220
Centrifugation machine	Remi instrument Ltd.
Spectro photometer	Hi media pvt. ltd
incubator	Hi media pvt. ltd
Elisa plate reader	Hi media pvt. ltd

- **Selection of the plant:**

  Based on literature review, the plant *Trachyspermum ammi* (Ajwain) was chosen for itsanti-ulcer activity.

- Procurement and authentification of the drug:

  Ethanolic extract of *Trachyspermum ammi* bark (Ajwain) was procured and authentificatefrom the Shamantak Enterprises Hinjawadi Pune.

A. Phytochemical analysis:[16]

The phytochemical tests were carried out for the above-mentioned plant extract usingthe standard procedures to identify the components Mentioned at the below;

a. Test for alkaloids

1. Dragendorff's Test:

Dragendorf's reagent gives a reddish-brown precipitate with plant extract andconfirms that test as positive. (Potassium bismuth iodide solution).

2. Wagner's Test:

Wagner's reagent gives a reddish-brown precipitate confirms that test as positive.(Solution of Iodine in potassium Iodide)

3. Mayer's Test:

Mayer's reagent gives a white creamy precipitate confirms that test as positive.(Potassium mercuric iodide solution).

b. Tests for carbohydrates:

1. Fehling's test (free reducing sugars):

At the first step equal volume of Fehling's A (copper sulphate in distilled water) and Fehling's B (potassium tartarate and sodium hydroxide in distilled water) reagents are mixed carefully. Then few drops plant extract was added and boiled. Brick red precipitate of cuprous oxide indicated the presence of free reducing sugars.

2. Molisch's test:

Treat 0.5 ml of plant extracts few drops of alcoholic α-naphtol was added.

Then 0.2 ml of concentrated sulphuric acid was added slowly along the sides of test tubes. Reddish-violet ring at the junction of the two layers indicated the presence of carbohydrates.

c. Tests for flavonoids:

1. Shinoda's test:

Treat 0.5 ml of plant extracts a piece of metallic magnesium was added, followed by addition of 2 drops of concentrated hydrochloric acid. Presence of deep red colouration indicated the presence of flavonoids in the extract.

1. Ferric chloride test:

Treat 0.5 ml of plant extracts a few drops ferric chloride solution was added. The presence of green colouration indicated the presence of flavonoids.

2. Alkaline reagent test:

Treat 0.5 ml of plant extracts few drops of sodium hydroxide solution was added. Ayellow colouration which turns to colorless by addition of few drops of dilute aceticacid indicated the presence of flavonoids.

d. Tests for glycosides:

1. Borntrager's test (Anthraquinone Glycosides):

Treat 1 ml of benzene and 0.5 ml of dilute ammonia solution were added to the plantextracts. A reddish pink colour indicated the absence of glycosides.

2. Keller killaini's test (Cardiac glycosides):

Treat 0.4 ml of glacial acetic acid containing traces of ferric chloride and 0.5 ml ofconcentrated sulphuric acid were added to the plant extracts carefully. A reddish- brown colour formed at the junction of the two layers and the upper layer turned bluish green indicating the absence of glycosides.

e. Test for resins:

Treat 0.5 ml of plant extracts were treated with a few drops of acetic anhydride solutionfollowed by one ml of concentrated sulphric acid. Resins give colouration ranging fromorange to yellow.

f. Tests for steroids and triterpenoids:

1. Liebermann - Burchard Test:

0.5 ml of plant extracts was treated with few drops of acetic anhydride, boil and cool, concentrated sulphuric acid is added along the sides of the test tube, shows brown ring at the junction of two layers and the upper layer turns green that showsthe presence of sterols and formation of deep red colour indicated the presence of triterpenoids.

B. In vitro Antioxidant activity:

a) In vitro Antioxidant activity by DPPH (96 well method) [17]:

➢ **Principle:**

1, 1 Diphenyl 2- Picryl Hydrazyl is a stable (in powder form) free radicalwith red color which turns yellow when scavenged. The DPPH assay uses thischaracter to show free radical scavenging activity. The scavenging reaction between (DPPH) and an antioxidant (HA) can be written as, DPPH-H + (A)(DPPH) + (H-A) Antioxidants react with DPPH and reduce it to DPPH-H and as consequence the absorbance decreases. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts interms of hydrogen donating ability.

➢ **Procedure:**

Antioxidant activity in the sample compounds was estimated for their free radical scavenging activity by using DPPH (1, 1-Diphenyl-2, Picryl-Hydrazyl) free radicals (George et al., 1996). 100µL of test compounds water were taken in the micro titer plate. 100µL of 0.1% methanolic DPPH was added over the samples carbon dots at different concentration (10, 20, 50µg/ml) and incubated for 30 minutes in dark condition. The samples were then observed fordiscoloration; from purple to yellow and pale pink were considered as strong and weak positive respectively and read the plate on Elisa plate reader at 490nm Radical scavenging activity was calculated by the following equation:

DPPH radical scavenging activity (%) =

[(Abs. of control – Abs. of test sample) / (Abs. of control)] x 100.

b) In vitro Antioxidant activity by H2O2 scavenging activity: [18, 19]

➢ **Principle:**

This H2O2 scavenging activity is bases on the reaction of ferrous ion (Fe+2) with sample. Ferrous ion specifically forms red orange sample complexwhich absorbs maximally at 508-510nm and this assay has been used for a longtime for quantitative measurement of iron in various samples

➢ **Procedure:**

Antioxidant activity in the sample compounds was estimated for their free radical scavenging activity by using H2O2 free radical by Ruch et al (1989). A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Extracts (100 µg/mL) in distilled water were added to a hydrogen peroxide solution (0.6 mL, 40mM). Absorbance of hydrogen peroxide at 230 nm was determined 10 minutes later against a blank solution containing the phosphate buffer without hydrogen peroxide.

H2O2 radical scavenging activity (%) =

[(Abs. of control – Abs. of test sample) / (Abs. of control)] x 100.

C. Assessment of In-Vitro Anti-Ulcer Activity:

Acid Neutralizing Capacity: [20,21]

➢ **Principle:**

The acid neutralization capacity (ANC) of an antacid is the amount of acid that neutralize, and it has been measured by a process known as back titration.

➢ **Procedure:**

The acid neutralizing capacity value for sample (200mg/ml, 400mg/ml,600mg/ml, 800mg/ml and 1000mg/ml was compared with the standard antacid Aluminium hydroxide + Magnesium hydroxide (500mg). To the 5ml quantityof this sample, water was added to make up the total volume 70ml and then mixed for one minute. There after 30ml of 1.0N HCl was added into standard and test preparation and stirred for 15minutes, drops of phenophthalein solution was added and mixed. The excess HCl was immediately titrated with0.5N Sodium hydroxide solution drop wise until a pink color is attained. The moles of acid neutralized is calculated by,

Moles of acid neutralized =

(vol. of HCl ×Normality of HCl) - (vol. Of NaOH × Normality of NaOH)

Acid neutralizing capacity (ANC) per gram of antacid =

Moles of HCl neutralized/ Grams of Antacid/Extract

H+/K+ - ATPase Inhibition Activity: [22,23,24]

➢ **Principle:**

The gastric hydrogen potassium ATPase or H^+/K^+ ATPase is the protonpump of the stomach. It exchanges potassium from the intestinal lumen with cytoplasmic hydronium and is the enzyme primarily responsible for the acidification of the stomach contents and the activation of the digestive enzyme pepsin.

- **Preparation of H+/K+ - ATPase Enzyme:**

To prepare H+, K+-ATPase enzyme sample, fresh sheep stomach was obtained from a local slaughterhouse. The stomach was cut opened, the mucosaat gastric fundus was cut-off, and the inner layer was scraped out for parietal cells.

Thus, obtained cells were homogenized in 16 mM Th (pH 7.4) containing 10%. Triton X-100 and centrifuged at 6000 g for 10 min. The supernatant (enzyme extract) was used to determine the H+, K+-ATPase inhibition.

- **Assessment of H+, K+-ATPase inhibition:**

The reaction mixture containing 0.1 ml of enzyme extract (300 µg) andplant extract at different concentrations (20, 40, 60, 80, 100µg/ml) was pre- incubated for 60 min at 37°C. The reaction was initiated by adding substrate 2mM ATP (200 µL), in addition to this 2 mM MgCl2 (200 µL) and 10 mM KCl(200 µL) was added. After 30 min of incubation at 37°C, the reaction was stopped by the addition of assay mixture containing 4.5% ammonium molybdate and 60% perchloric acid followed by centrifugation at 2000 g for 10 min. Inorganic phosphate released was measured spectrophotometrically at660 nm. Enzyme activity was calculated as micromoles of per-incubatedreleased for an hour at various doses (0-100 µg) of sample VS.

Results were compared with the known antiulcer PPA inhibitor drugomeprazole.

Percentage of enzyme inhibition was calculated by using the formula;

Percentage of inhibition=

[Activity (control) – Activity (test)/Activity (control)] × 100

- **Determination of urease inhibitory assay:** [25,26]

- **Principle:**

Urea is common metabolic waste product of protein digestion in most vertebrates that is toxic to most living organism. Urease catalyses the breakdown of urea into ammonia and carbondioxide. The test organism is cultured in a medium containing urea and the indicator phenol red. If the bacterial strain is urease-producing, the enzyme will hydrolyse the urea to giveammonia and carbondioxide. With the release of ammonia, the medium becomealkaline shown by change in color of indicator to reddish pink.

- **Procedure:**

The reaction buffer (10 µL of 50 mM K2HPO4 buffer [pH = 7.0] and 10µL of ddH2O) were added to each well of a 96-well plate. Jack bean urease (JBU, 1 µg µL− 1) was freshly prepared with 50 mM phosphate buffer. Then, 20 µL of the enzyme and 10 µL of the tested inhibitor (2.5–20 µg µL− 1) were poured in the reaction mixture. The mixture was incubated at 37◦C for 10 min.Enzymatic reactions were started after the addition of 40 µL of the urea substrateand incubation at 37◦C for 10 min.

RESULTS and DISCUSSION

A. Pharmacognostic characteristics of *Trachyspermum ammi* bark extract:

Table no. 1: Pharmacognostic characteristics of ethanolic extract of *Trachyspermum ammi* bark:

Sr. no.	Test	Observation	Standard
	Grade	**G/100**	**G/100**
1.	Description (Sensoryevaluation)	Black colour	Black colour
2.	Odour & taste	Aromatic	Aromatic
3.	Solubility	Miscible	Miscible
4.	% moisture	2.24%w/w	1-3%w/w
5.	% Ash content (As per I. P.)	2.55%w/w	2-7%w/w
6.	Nature	Viscus	Viscus

B. Phytochemical analysis:

Table no.2: Phytochemical analysis of ethanolic extract of *Trachyspermumammi* bark.

Sr. No.	PhytochemicalConstituents	Tests	Results
1.	Test for alkaloids	Dragendorff's Test	+++
		Wagner's Test	+++
		Mayer's Test	-
2.	Tests for carbohydrates	Fehling's test	+++
		Molisch's test	+++
3.	Tests for flavonoids	Shinoda's test	+++
		Ferric chloride test	+++
		Alkaline reagent test	+++
4.	Tests for glycosides	Borntrager's test	-
		Keller killaini's test	-
5.	Test for resins		+++
6.	Tests for steroids and triterpenoids	Liebermann Burchard Test	+++
		Salkowski Test	+++
7.	Tests for tanins	Ferric chloride Test	+++
8.	Tests for phenolic	Ferric chloride Test	+++
9.	Tests for amino acids	Millons Test	-
		Ninhydrin Test	-
10.	Tests for protein	Biuret Test	-

C. In-vitro Antioxidant activity:

a) DPPH free radical scavenging activity:

Table No.3: Effect of ethanolic extract of *Trachyspermum ammi* bark by using DPPH free radical scavenging activity (96 well method).

Sr. No	Concentration (µg/ml)	Absorbance (std)	% inhibition (std)	Absorbance (extract)	% inhibition (extract)
1	200	0.480±0.036 ***	75.28	0.588±0.009 ***	69.72
2	400	0.337±0.016 ***	80.58	0.546±0.012 ***	71.88
3	600	0.281±0.013 ***	85.53	0.433±0.025 ***	77.70
4	800	0.230±0.010 ***	88.15	0.318±0.024 ***	83.62
5	1000	0.125±0.018 ***	93.56	0.216±0.013 ***	88.87
	Control	-	-	1.942	-

Values are in mean ±S. E. M. (n = 3); all values compared with Control by usingOne-way (ANOVA) followed by Dunnett's "t" test.

Ns - non significant

* - Significant p<0.05

** - Moderately Significant p<0.01

*** - Highly Significant p<0.00

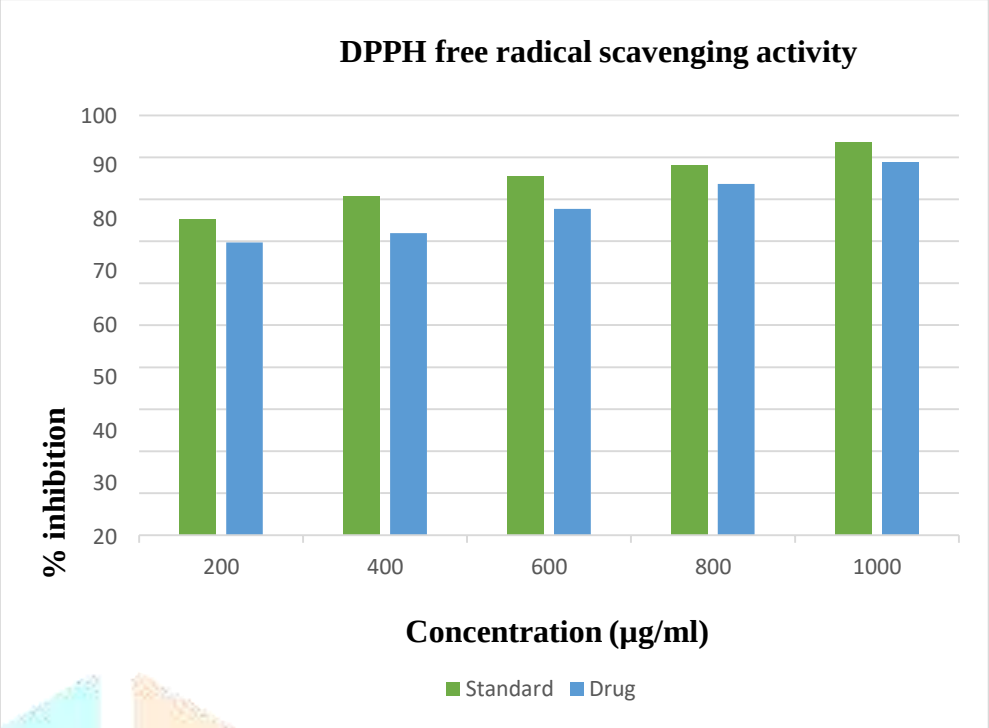

Fig no.2: DPPH free radical scavenging activity of ethanolic extract of *Trachyspermum ammi* bark at different concentrations.

b) H2O2 free radical scavenging activity:

Table No.4: Effect of ethanolic extract of *Trachyspermum ammi* bark by usingH2O2 free radical scavenging activity

Sr. No.	Concentration (µg/ml)	Absorbance (std)	% inhibition (std)	Absorbance (extract)	% inhibition (extract)
1.	200	0.512±0.022 ***	73.65	0.553±0.004 ***	71.54
2.	400	0.296±0.012 ***	84.76	0.469±0.015 ***	75.87
3.	600	0.224±0.009 ***	88.43	0.385±0.007 ***	80.23
4.	800	0.183±0.0012 ***	90.53	0.278±0.006 ***	85.77
5.	1000	0.111±0.004 ***	94.32	0.185±0.006 ***	90.43
6.	Control	-	-	1.942	-

Values are in mean ±S. E. M. (n = 3); all values compared with Control by using One-way (ANOVA) followed by Dunnett's "t" test.

Ns - non significant

* - Significant p<0.05

** - Moderately Significant p<0.01

*** - Highly Significant p<0.001

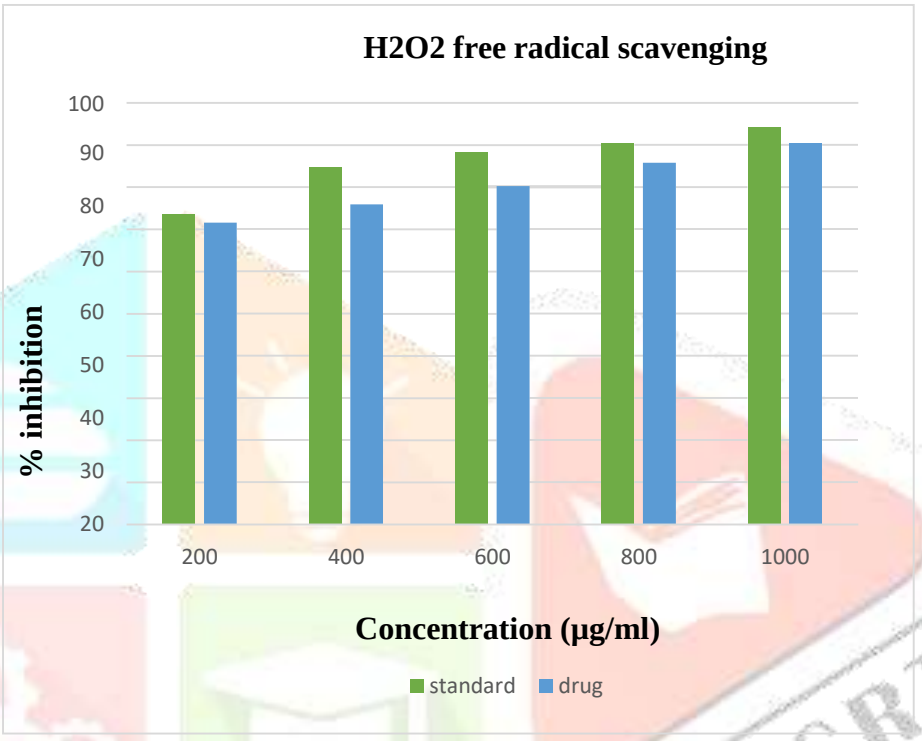

Fig no.3: H2O2 free radical scavenging activity of ethanolic extract of *Trachyspermum ammi* bark at different concentrations.

D. Assessment of in-vitro anti-ulcer activity:

a. Acid neutralizing capacity:

Table No.5: Effect of ethanolic extract of *Trachyspermum ammi* bark on Acidneutralizing capacity.

Sr. No.	Concentration(µg/ml)	Volume ofNaOH consumed (ml)	Moles of Acid Consumed	ANC per gram of Antacid
1.	200	8.5	25.75	128.75±9.16*
2.	400	5.2	27.4	68.5±10.67***
3.	600	4.3	27.85	46.41±8.68***
4.	800	3.5	28.25	35.31±2.62***
5.	1000	6.5	26.75	26.75±4.42***
6.	200	17.8	21.1	105.5±4.45*
7.	400	13.6	23.2	58.00±6.68***
8.	600	10.5	24.75	41.25±2.89***
9.	800	9.9	25.05	41.75±3.034***
10.	1000	8.8	25.6	25.6±3.23***

Values are in mean ±S. E. M. (n = 3); all values compared with Control by using One-way (ANOVA) followed by Dunnett's "t" test.

Ns - non significant

* - Significant $p<0.05$

** - Moderately Significant $p<0.01$

*** - Highly Significant $p<0.001$

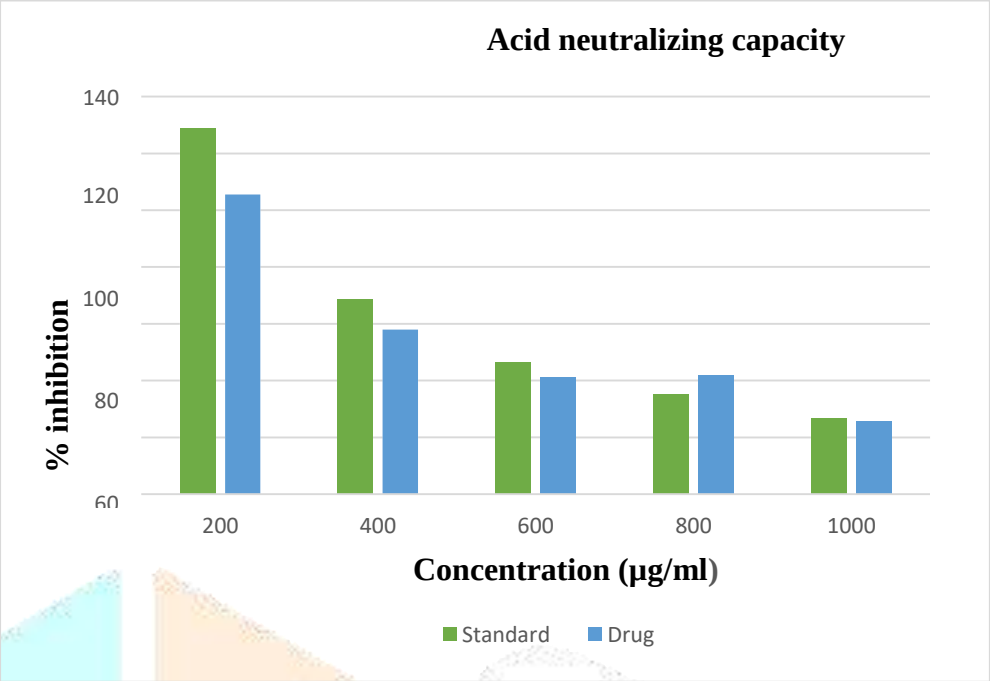

Fig.no.4: Acid neutralizing capacity of ethanolic extract of *Trachyspermum ammi* bark at different concentrations.

b. Assessment of H+/K+-ATPase inhibition:

Table No.6: Effect of ethanolic extract of *Trachyspermum ammi* bark on H + /K+ ATPase inhibition activity.

Sr. no	**ıcentration (µg/ml)**	**bsorbance (extract)**	**orbance(std)**	**% inhibition (extract)**	**% inhibition (std)**
1.	20	0.35±0.057*	0.20±0.41*	39.65	65.51
2.	40	0.30±0.037*	0.18±0.014*	48.27	68.96
3.	60	0.28±0.053*	0.15±0.029*	51.72	74.13
4.	80	0.27±0.045*	0.12±0.021*	53.44	79.19
5.	100	0.22±0.043*	0.09±0.08*	62.06	84.48
	Control	0.58	-	-	-

Values are in mean ±S. E. M. (n = 3); all values compared with Control by using One-way (ANOVA) followed by Dunnett's "t" test.

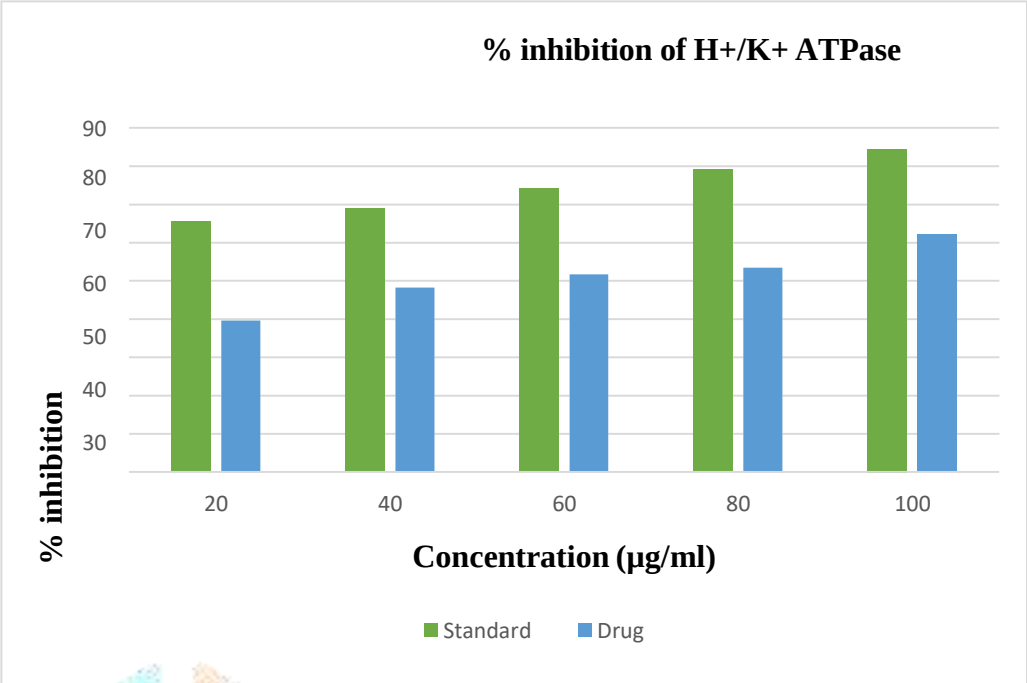

Fig.no.5: H + /K+ ATPase inhibition activity of ethanolic extract of *Trachyspermumammi* bark at different concentrations.

c. Urease inhibition assay:

Table No.7: Effect of ethanolic extract of *Trachyspermum ammi* bark on ureaseinhibition assay.

Sr. no	ıcentration (µg/ml)	bsorbance (extract)	orbance(std)	% inhibition (extract)	% inhibition (std)
1.	10	0.363±0.0114	0.276±0.0016	48.87	72.12
2.	100	0.284±0.004	0.269±0.0186	60.0	72.76
3.	500	0.284±0.008	0.293±0.0044	65.35	77.76
4.	1000	0.215±0.007	0.184±0.0074	69.71	81.38
5.	Control	0.710	-	-	-

Values are in mean ±S. E. M. (n = 3); all values compared with Control by using One-way (ANOVA) followed by Dunnett's "t" test.

Ns - non significant * - Significant

* - Significant $p<0.05$

** - Moderately Significant $p<0.01$

*** - Highly Significant $p<0.001$

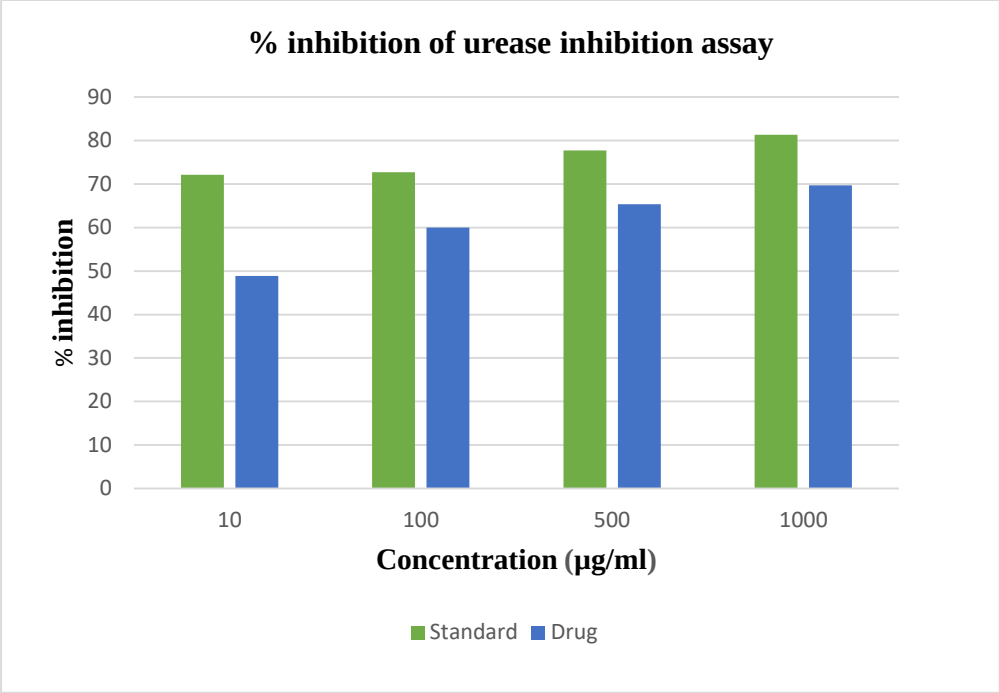

Fig no.6: Urease inhibition assay of ethanolic extract of *Trachyspermum ammi* bark at different concentrations.

DPPH is a stablefree radical, which changes its colour from violet to yellow upon reduction by the process of electron donation. Ethanolic extract of *Trachyspermum ammi* bark when reacts with DPPH free radical, convert it to 1,1-diphenyl-2-(2,4,6-trinitophenyl) hydrazine. The scavenging potential of the antioxidants present in ethanolic extract of *Trachyspermum ammi* bark can thus be determined by their degree of discoloration to yellow.

In-vitro Antioxidant activity by DPPH free radical scavenging activity of ethanolic extract of *Trachyspermum ammi* bark by using different concentrations as (200µg/ml, 400µg/ml, 600µg/ml, 800µg/ml, 1000µg/ml). From the results it is clear thatthe scavenging effect of DPPH radical increased with the increasing concentration of ethanolic extract of *Trachyspermum ammi* bark than compared with Ascorbic acid as standard. The ethanolic extract of *Trachyspermum ammi* bark exhibited highly significant activity in a dose dependent manner. Percentage of inhibition level was within range of 69.72% to 88.87%. As the concentration increases the percentage of inhibition also increases. At 1000 µg/ml it shows maximum percentage of inhibition and that is 88.87%.

Scavenging of OH - is an important antioxidant activity because of its very highreactivity, which can easily cross the cell membranes at specific sites, react with most biomolecules and furthermore cause tissue damage and cell death. Thus, removing OH is very important for the protection of living systems. In-vitro Antioxidant activity by H2O2 free radical scavenging activity of ethanolic extract of *Trachyspermum ammi*bark at a various concentration as (200µg/ml, 400µg/ml, 600µg/ml, 800µg/ml, 1000µg/ml) revealed dose dependent manner when compared with Ascorbic acid as standard.

The ethanolic extract of *Trachyspermum ammi* bark showed highly significantactivity in a dose dependent manner. Percentage of inhibition level was within range of 71.54 % to 90.43 %. As the concentration increases the percentage of inhibition also increases. At 1000 µg/ml it shows maximum percentage of inhibition that is 90.43%.

Anti-ulcer activity of ethanolic extract of *Trachyspermum ammi* barkwas carried out by Acid Neutralizing Capacity, H+/K+ - ATPase Inhibition Activity and urease inhibitory assay. In Acid Neutralizing Capacity, the acid neutralizing effect of the ethanolic extract of *Trachyspermum ammi* bark was studied by using different concentration as (200µg/ml, 400µg/ml, 600µg/ml, 800µg/ml, 1000µg/ml) and standard Aluminium Hydroxide + Magnesium Hydroxide [Al (OH)3 + Mg (OH)2] (200 µg/ml). The resultsobtained envisage that the ethanolic extract of *Trachyspermum ammi* bark showed a significant at 200 mg concentration and highly significant at concentration 400mg, 600mg, 800mg, and1000mg reduction in acid neutralizing capacity (ANC), i.e., 105.5,58.00, 41.25, 41.75 and 25.6 respectively.

In the Urease inhibition assay, the ethanolic extract of Trachyspermum ammi bark at different concentration (10 µg/ml, 100 µg/ml,500 µg/ml,1000 µg/ml) showed inhibition of urease enzyme when compared with the Thiourea as a standard. The ethanolic extract of Trachyspermum ammi bark showed highly significant activity. Percentage of inhibition level was within range of 48.87% to 69.71%. As, the concentration increases the percentage of inhibition also increases. At 1000 µg/ml it shows maximum % inhibition is 69.71%.

CONCLUSION:

Whole of the pharmaceutical industry is now paying consideration towards design and development of new indigenous plant-based drugs through searching of leads from traditional system of medicine. Recent years, ethnobotanical and traditional uses of naturalcompounds, especially of plant origin received much interest as they are scientifically tested for their effectiveness and generally believed to be harmless for human use. It is bestconventional approach in the search of new molecules for management of several diseases.Detailed screening of literature available on *Trachyspermum ammi* showed the fact that itis a popular remedy among the various ethnic groups and traditional practitioners for treatment of different types of ailments. In the present study, results indicate that the ethanolic extract of *Trachysprmum ammi* bark possess different phytochemicals such as polyphenolic compounds as alkaloids, flavonoids, tannins, steroids, and phenols, resins, carbohydrates. According to the phytochemical analysis of ethanolic extract of *Trachyspermum ammi* bark the results showed that alkaloids, carbohydrates, flavonoids, resins, triterpenoids, tanins and phenolic compounds are present in higher amount, while amino acids and proteins are totally absent.

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