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A STUDY ON ANTIOXIDANT AND GASTRO-PROTECTIVE EFFECTS OF *TRACHYSPERMUM AMMI* LINN EXTRACT USING DIFFERENT IN-VITRO TECHNIQUES

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ABSTRACT

Herbal medicine also called botanical medicine or phytomedicine refers to using a plant's seeds, berries, roots, leaves, bark, or flowers for medicinal purposes. Herbalism has a long tradition of use outside of conventional medicine. It is becoming more main stream as improvements in analysis and quality control along with advances in clinical research show the value of herbal medicine in the treating and preventing disease. The use of local plants in folk medical practices has a long history. The resource base of the traditional medical practices prevalent in rural and tribal villages of India and abroad is mainly the plants. *Trachyspermum ammi* which is commonly known as Ajowan. Among traditional potential herbs used as spice in day to day life, ajwain (*Trachyspermum ammi* L.) belonging to family Apiaceae. The phenolic compound in fruits, vegetables, herbs and spices possess potent antioxidant, anti-inflammatory, antigenotoxic and anticancer activities. The dried seeds of *Trachyspermum ammi* were extracted with methanol using a Soxhlet extractor. The total phenolics content of seed as determined by Fenton reaction and was found to be good antioxidant activity as different dose concentrations. The antioxidant activity of seed extract was carried out with ascorbic acid as a standard reducing agent. The present results were made with the use of UV-Visible Spectrophotometer. In this plant Trachyspermum ammi Extract there was a remarkable concentration dependent free radical scavenging and reducing power was exhibited. We were also found the gastroprotective activity of *T. ammi* extract as compared to Liv52 standard drug and control liver enzymes was also significant using SGOT and SGPT enzymes. *T. ammi* extract treated histology slide of the liver a classic liver lobule

the hepatocytes are polyhedral with eosinophilic cytoplasm and a usually central nucleus. Between the cords of hepatocytes are endothelially lined sinusoids showing normal histology of liver as compared to control and Liv52 treated groups and significant reduction were also found to intestine histology as compared to untreated control. In conclusion the present study indicates that *Trachyspermum ammi* extract may be a potential source of natural antioxidant and gastroprotective effect. Trachyspermum ammi appears as an excellent option to enrich the diet with antioxidant phenolic compounds as well as gastroprotective agent.

KEYWORDS: Gastro-protective, Antioxidant activity, Fenton Reaction, Hydroxyl radical, *Trachyspermum ammi*, SGOT and SGPT enzymes.

1. INTRODUCTION

Traditional therapeutic uses of *T. ammi* fruits include: galactogogue, stomachic, carminative (Chialva *et al* 1993), Expectorant, Antiseptic (Choudhury, *et. al.*, 1998), Amoebiasis, Antimicrobial, seeds fried in oil and used as a thin soup as a galactogogue (Howard, *et. al.*, 1985), used in curing diarrhoea, Parasiticidal, and given in treatment of amenhorroea (Shome, *et. al.*, 1996), Bronchitis, colic pain (Singh, et. al., 2003), Antipyretic, febrifugal (Vedavathy, and Rao, 1995). The seed of ajwain is bitter, pungent, and it acts as anthelmintic, carminative, laxative, and stomachic. It also cures abdominal tumors, abdominal pains, and piles (Krishnamoorthy and Madalageri, 1999). Catecholamines from the adrenal medulla would have contributed either to a stress associated release of ACTH or to a rise in intracellular cyclic AMP and catecholamine release is associated with enlargement of adrenal gland (Berne, *et. al*., 1988). Presence of terpenes, glycosides and sterols in plant has been found to exert active antiinflammatory effects (Chawla, *et. al*., 1987). Intragastric application of absolute ethanol has long been used as a reproducible method to induce gastric lesions in experimental animals (Szabo, *et. al.*, 1981). Gastric lesion is accompanied with the formation of free radicals (FRs) and reactive oxygen species (ROS) (Cho, *et. al.*, 1991; Lutnicki, *et. al*., 1992; Bast, *et. al.*, 1991). These radicals in particular seem to play an important role in ulcerative and erosive lesions of the gastrointestinal tract (Hirokawa, *et. al.*, 1998), as they attack and damage many biological molecules. Therefore, treatment with antioxidants and FR scavengers can decrease ethanolinduced gastric mucosal damage (La Casa, *et. al*., 2000; Tahir, *et. al.*, 1993). Natural products, including plant drugs, have been used as gastroprotective agents all over the World. The natural products-derived therapeutic agents include the terpenes glycyrrhetic acid and sodium ecabet (Farina, *et. al.*, 1998; Onoda, *et. al*.,

2010). Several plant drugs are used in Latin American traditional medicine to treat symptoms related with gastric ulcers. Therefore, we have planned to carry out the antioxidant and gastro protective effect of *Trachyspermum ammi* extract using different In-vitro techniques.

2. MATERIALS AND METHODS

Plant material – *Trachyspermum ammi* seed was collected from Local Market, Raipur (Chhattisgarh), India.

Chemicals and Reagent samples – The reagents used were of highest purity (>99.95%) and were purchased from Sigma Chemical Co. (Germany) and other. Sample absorbances were read using a Lambda 532 nm, UV Spectrometer made by Varian.

Preparation of extract - Dried powdered of *Trachyspermum ammi* seed (15 g) were extracted by continuous mixing in 500 ml 50% methanol and water, 24 h at room temperature. After the filtration process, methanol was evaporated until only water remained through evaporation on water bath at 60-70 0c temperature. The final extract was kept in air tied box.

Deoxyribose assay to assess OH -radical scavenging activity

The OH- radical scavenging activity of *Trachyspermum ammi seed* extract (10–100 ug/ml) was determined according to the deoxyribose method reported of Halliwell, *et al*., (1987). In the protocol the presence of 100 lM EDTA. $FeCl_3$, H_2O and ascorbic acid were prepared in degassed H_2O prior to use. The reaction tube contained (final concentrations) 3.6 mM deoxyribose, 100 lM EDTA, 1 mM H2O2, 100 lM L- ascorbic acid, 100 lM $FeCl_3$, H_2O in 25 mM phosphate buffer, pH 7.4 in 1.0 ml total volume. Samples was kept in incubation at 38º C, 1 hrs, 1.0 ml 1.0% TBA in 0.05 M NaOH and 1.0 ml 10% TCA were added to the reaction mixture after that samples was heated in a boiling water bath for 15 min. After the samples were cooled, the absorbances were read at 532 nm. The IC50 value of the plant extract was compared with that of ascorbic acid, which was used as the standard. The result of lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The percentage of inhibition of hydroxyl radical was calculated as follows:

$$\% \text{ Inhibition} = \text{Abs: } \frac{\text{532 nm Control Abs. - 532 nm sample Abs.} \times 100}{\text{532 nm Control Abs}}$$

Antioxidant capacity of test compounds was expressed as IC_{50}, the concentration necessary for 50% inhibition concentration of TBARS.

Gastro-protective Activity: Induce the gastric problems: We have chosen the NSAID drug for the induction of abnormalities in all animals. After induction, we have divided these animals in to 3 groups.

- NSAID treated as control.
- Liv52 treated as Positive control.
- *Trachyserumum ammi* treated (250 and 500 mg/kg).

1. Untreated Control: This group consisted of six animals induced gastric problems using NSAID drug. During the treatment, the body weight was measured and biochemical activity was performed using SGOT and SGPT serum enzymes.

2. Liv52 treated as Positive control: This group consisted of six animals induced gastric problems using NSAID drug after the induced gastric problem follow-up the treatment of Liv52 (250 mg/kg) drug as a positive control. During the treatment, the body weight was measured and biochemical activity was performed using SGOT and SGPT serum enzymes.

3. *Trachyserumum ammi* treated: This group consisted of six animals induced gastric problems using NSAID drug after the induced gastric problem follow-up the treatment of *Trachyserumum ammi* extract (250 and 500 mg/kg). During the treatment, the body weight was measured and biochemical activity was performed using SGOT and SGPT serum enzymes. The animals were dissected at the intestine and liver was taken out. The observation was made by the microscopic studies.

Serum biochemical analysis

The activity of SGOT and SGPT was carried out by the method of Reitman and Frankel, (1957) and expressed as IU/L. The collected data were statistically analysed by student's t-test and the treatment groups were considered statistically significant at $P<0.05$.

3. RESULT

Antioxidant Activity

The results of the effects of the examined *Trachyserumum ammi* extract as well as control solutions on OH- radical production. They show that all extract of *Trachyserumum ammi extract* and control solutions as a ascorbic acid inhibited the production of OH- radicals. The % of free racial scavenging activity of methanolic extract of *Trachyserumum ammi* extract

presented in Table 1 have reducing power, the free radial OH- scavenging activity of the extract increases with increasing the concentration.

Table 1: Antioxidant activities of *Trachyserumum ammi* extract using Fenton reaction.

Concentration (in µl)	Ascorbic acid (Mean + SE)	*Trachyserumum ammi* extract (Mean + SE)
10	11.12 ± 1.90	9.54 ± 0.165
20	22.20 ± 3.01	13.25 ± 0.78
30	38.57 ± 1.51	34.66 ± 0.44
40	46.72 ± 1.15	46.78 ± 0.78
50	51.33 ± 1.25	49.25 ± 1.12
60	59.03 ± 1.00	52.55 ± 1.18
70	68.03 ± 0.91	59.74 ± 0.15
80	78.59 ± 1.01	67.12 ± 0.12
90	86.44. ± 1.41	76.78 ± 0.34
100	94.34 ± 0.87	90.12 ± 0.345

Gastro-protective Activity

Cough Reflexes: The expiratory effort due to an endotracheal mechanical stimulus was reduced by *Trachyserumum ammi* extract shows the dose-response in SGOT and SGPT enzyme as compared to NSAID treated group. The mortality rate was observed to be nil in all experimental groups. A significant reduction in body weight gain. Serum SGOT and SGPT concentration showed a significant increase as compared to control (Table 2).

Table 2: Effect of *Trachyserumum ammi* extract on cough forming activity using SGOT and SGPT enzyme.

S.N.	Groups	Body Weight		SGOT	SGPT
		Initial	Final		
1.	Untreated control	22.03±0.62	23.03±0.26	72.54±0.53	76.01±0.77
2.	Positive control (NSAID treated)	21.56±0.47	19.86±0.69	52.85±.0.56	57.11±0.55
3.	Liv52 treated	21.77±0.63	23.03±0.75	71.12±0.85*	74.23± 0.47*
4.	*Trachyserumum ammi (250mg/kg)*	22.16±0.53	23.06±0.62	62.47± 0.51*	64.49± 0.56*
5.	*Trachyserumum ammi (500 mg/kg)*	21.23±0.49	22.56±0.54	66.46± 0.53*	68.19± 0.41*

*denotes statistically significant as compared to positive control group at $p<0.05$.

Histology of Liver

Result shows treated group histology slide of the liver the anatomical lobule with the central vein in the center is useful for the study of liver morphology, but doesn't give much information about the functional units of the liver. With the portal triad in the center of the

functional lobule and blood flowing from this center in all directions toward various "central veins" some liver disease can be better explained. For example reduced arterial blood supply would manifest itself initially by affecting the cells nearest the central vein while toxic substances in the blood would show up first in cells near the portal triad. Liver macrophages, called Kupffer Cells, are present in sinusoids. Result shows treated and without treatment of *T. ammi* extract histology slide of the intestine the total, damage, normal and cript cells for the study of intestine morphology.

Histology of Intestine

S.N.	Groups	Micro villi in %			Cript Cells
		Total	Damage	Normal	
1	Control (treated with NSAID)	41	29.5	11.5	77.5
2	Liv52 Positive Control	42	22.25	19.75	84.0
3	*T. ammi* extract (250 mg/kg)	40.4	24.6	15.8	77.6
4	*T. ammi* extract (250 mg/kg)	42.4	26.4	16.0	78.4

4. DISCUSSION AND CONCLUSION

Plants had been used for medicinal purposes long before recorded history. Ancient Chinese and Egyptian papyrus writings describe medicinal uses for plants. Indigenous cultures (such as African and Native American) used herbs in their healing rituals, while others developed traditional medical systems (such as Ayurveda and Traditional Chinese Medicine) in which herbal therapies were used. Researchers found that people in different parts of the world tended to use the same or similar plants for the same purposes (Birks, *et. al.*, 2007).

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We were also found the gastroprotective activity of *T. ammi* extract as compared to Liv52 standard drug and control liver enzymes was also significant using SGOT and SGPT enzymes. *T. ammi* extract treated histology slide of the liver a classic liver lobule the hepatocytes are polyhedral with eosinophilic cytoplasm and a usually central nucleus. Between the cords of hepatocytes are endothelially lined sinusoids showing normal histology

of liver as compared to control and Liv52 treated groups and significant reduction were also found to intestine histology as compared to untreated control.

Early animal's studies were also demonstrated that, an important gastroprotective effect was shown in studies on several experimental ulcer models in rats using aqueous extract of *Neem (Aradirchta indica)* bark (Bandyopadhyay, *et. al*., 2002), ethanol extract of *Aqeratum conyzoides* (Shirwaikar, *et. al*., 2003), *Bacopa monniera* and *Azadirachla indica* extract (Dorababu, *et. al*., 2004).

This study provides an evidence that extracts originating from the plants used in ancient herbal medicine appear to contain highly effective, most likely flavonoids, that are capable of protecting the gastric problems and possible useful in the therapy of acute and chronic gastric problems. So the extract would be a good alternative for broad spectrum antibiotic, antioxidant and gastroprotective effect.

Disputed interests

There were no declared conflicts of interest by the authors.

REFERENCES

1. Bandyopadhyay, U, Biswas. K, Chatterjee, R, Badyopadhyay, D, *et. al.*, Gastroprotective effects of Neem (*Azadirachta indica*) bark extract: possible involment of H(+)-K(+)-ATPase inhibition and scavenging of hydroxyl radical. *Life Sci.*, 2002; 71(24): 2845-2865.
2. Bast, A., Haenen, G.R., Doelman C.J. Oxidant and antioxidant, State of the *art. Am J. Med.*, 1991; 91: 2s13s.
3. Birks. J, Grimley. Evans. J. Ginkgo biloba for cognitive impairment and dementia. *CochraneDatabase Syst Rev*., 2007; 2: CD003120.
4. Chawla AS, Handa SS, Sharma AK, Kaith BS, Plant antiinflammatory agents, *J Sci Ind Res.*, 1987; 46: 214-223.
5. Chialva, F., Monguzzi, F., Manitto, P., Akgül, A., Essential oil constituents of Trachyspermum copticum (L.) Link fruits *Journal of Essential Oil Research*, 1993; 5(1): 105-106.
6. Cho CH, Pfeiffer, C.J, Misra, H.P., Ulcerogenic mechanism of ethanol and the action of sulphanilyl fluoride on the rat stomach in-vivo. J. Pharm Pharmacol, 1991; 43: 495-498.

7. Choudhury S, Riyazuddin A, Kanjilal PB Leclercq PA, Composition of the seed oil of *Trachyspermum ammi* (L.) Sprague from Northeast India, *Journal of Essential Oil Research*, 1998; 10(5): 588-590.
8. Dorababu M, Prabha T, Priyambada S, Agrawal VK, Aryya NC, Goel RK., Effect of *Bacopa monniera* and *Azadirachta indica* on gastric ulceration and healing in experimental NIDDM rats. *Indian J Exp Biol*, Apr, 2004; 42(4): 389-397.
9. Farina, C.; Pinza, M.; Pifferi, G., Synthesis and anti-ulcer activity of new derivatives ofglycyrrhetic, oleanolic and ursolic acids. *Farmaco*, 1998; *53*: 22-32.
10. Hirokawa M, Miura S, Yoshida H, Kurose I, Shigematsu T, Hokari R, Higuchi H, Watanabe N, Yokoyama Y, Kimura H, Kato S, Ishii H., Oxidative stress and mitochondrial damage precedes gastric mucosal cell death induced by ethanol administration. *Alcohol Clin Exp Res.*, 1998; 22: 111S-114S.
11. Howard MP, Reynolds RD, Moser PB, Andon MB, McConnell W Acharya S., Special Foods of High Nutritive Value Consumed by Nepalese Lactating Women, *Federation Proceedings*, 1985; 44(5): 1505.
12. Krishnamoorthy V, Madalageri MB, Bishop Weeds (*Trachyspermum ammi*) an essential crop for north Karnatka, *Journal of Medicinal and Aromatic Plant Sciences*, 1999; 21(4): 996–998.
13. La Casa C, Villegas I, Alarcon de la Lastra C, Motilva V, Martin Calero MJ, Evidence for protective and antioxidant properties of rutin, a natural fl avone, against ethanol induced gastric lesions. *J Ethnopharmocal* ., 2000; 71: 45-53.
14. Lutnicki K, Wrobei J, Ledwozyw A, Trebas- Pietrast E., The effect of calcium ions on the intensity of peroxidation processes and the severity of ethanol-induced injury to the rat's gastric mucosa. Arch Vet Pol, 1992; 32: 125-132.
15. Onoda, Y.; Takido, M.; Magaribuchi, T.; Tamaki, H., Effects of 12-sulfodehydroabietic acid monosodium salt (TA-2711), a new anti-ulcer agent, on gastric mucosal lesions induced by necrotizing agents and gastric mucosal defensive factors in rats. *Jpn. J. Pharmacol*, 1990; *52*: 631-638.
16. Singh, V.K., Singh S, Singh DK, Pharmacological effects of spices, In Recent Progress in Medicinal Plants, Phytochemistry and Pharmacology, Stadium Press, Houston Texas, USA, 2003; 2: 321-353.
17. Szabo S, Trier JS, Frankel PW., Sulfhydryl compounds may mediate gastric cytoprotection. *Science*., 1981; 214: 200-202.

18. Tahir KE, Ashour MM, al-Harbi MM., The respiratory effects of the volatile oil of the black seed (Nigella sativa) in guinea-pigs: elucidation of the mechanism(s) of action. *Gen Pharmacol*., 1993; 24: 1115-1122.
19. Vedavathy S, Rao DN, Herbal folk medicine of Tirumala and Tirupati region of Chittoor district, Andhra Pradesh, Fitoterapia, 1995; 66(2): 167-171.