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ANTICOAGULANT ACTIVITY OF ETHANOL AND AQUEOUS EXTRACTS OF *PETROSELINUM CRISPUM* MILL.

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ABSTRACT

This Study is designed to evaluate the Anticoagulant activity of *Petroselinum crispum* Mill. Anticoagulants are administered to patients with myocardial infarction, venous thrombosis, peripheral arterial emboli and pulmonary emboli and prevent transient ischemic attacks and to reduce the risk of recurrent myocardial infarction. The male wister albino rats were divided into 7 groups of Six animals. Group I served as a control. Group II rats were administered with Phytomena (10mg/kg) orally. Group III rats were administered with standard drug of Warfarin sodium (5mg/kg) orally. Group IV,V animals were administered with ethanol extract of *Petroselinum crispum* Mill. (200mg/kg & 400mg/kg respectively) orally. Group VI,VII animals administered with aqueous extract of *Petroselinum crispum* Mill. (200mg/kg,400mg/kg respectively) orally. After the end of the absorption time, they are anesthetized by intravenous injection of 60 mg/kg sodium pentobarbital. The retro-orbital bleeding method was employed to collect 1.8ml blood into a plastic syringe containing 0.2 ml 100 mM citrate buffer ph 4.5 (Behring werke, Marburg). The sample is immediately agitated and centrifuged in a plastic tube at 1500 g for 10 min. Plasma is transferred to another plastic tube and the coagulation test for the determination of thrombin time (TT), thromboplastin time (PT) and activated partial thromboplastin time (APTT)were performed within3 hr. Ethanol and aqueous extract of *Petroselinum crispum* mill leaves, whose anticoagulant effectiveness is comparable to that of its commercial counterpart, warfarin, (Group III) showed a positive inhibition of clot formation with significant prolonged a PTT, PT, TT activated time. In all Aqueous extract(400mg/kg) of *Petroselinum crispum* Mill. found to be significant and most effective than ethanol extracts in positive anticoagulation effect. In all, it is obvious that it can be used as cardioprotector.

Key words: Anticoagulant activity, Cardioprotector, *Petroselinum crispum* Mill., Warfarin sodium.

INTRODUCTION

Man and Medicinal Plants

There has always been a strong relationship between man and medicinal plants. Right from the start of civilization plants have been used as medicine by men and animals, probably by animals before men [1].

Phytopharmaceutical Research in India

Phytopharmaceuticals can be defined as drugs formulated using traditional compounds derived from botanicals (herbs or medicinal plants) instead of chemicals.

This present work and investigations were focused on the plant *Petroselinum crispum* Mill. and its cardio protective activity was carried out. *Petroselinum crispum* Mill. is a non-hairy biennial or short-lived perennial with a much branched stem. A thin, white, spindle-shaped root produces the erect, grooved, glabrous, angular stem that can reach a height of slightly over 2 feet.

The plant is often cultivated as an annual for its foliage, especially in California, Germany, France, Belgium and Hungary. There are numerous varieties. Parts used are the ripe fruits (seeds), the above-ground herb and the leaves. It contains a volatile oil (including about 20% myristicin, about 18% apiole, and many other terpenes), flavonoids, phthalides, coumarins (including bergapten), vitamins A, C, and E, and high levels of iron. The flavonoids are anti-inflammatory and antioxidant. The volatile oil relieves cramps and flatulence, and is a strong uterine stimulant [2-6].

In classical medicine, it fruits were used primarily as a stomachic or carminative (aids digestion and expels gas), and the leaves as a diuretic (increases flow of urine).

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The fruit also enjoyed some reputation as an emmenagogue and an abortifacient (stimulates menstrual flow and abortion). Although there may be some basis in fact for these uses of parsley, such attributes as a cure for diabetes, heart problems, liver ailments, and venereal disease are purely fanciful. The aim of research is to find out new drugs from indigenous plant which are potent drugs. These plants are traditional medicinal plants. Their chemical characters and their mode of action and toxicity studies are yet to be established. Present study deals with the phytochemical and pharmacological studies of *Petroselinum crispum* mill with special reference to cardio protective activity in animal models [7-11].

MATERIALS AND METHODS

Plant Material

The leaves of *Petroselinum crispum* Mill. Plants were collected from the field orange valley in Masakal village, The Nilgiris. The plant was then authenticated by Mrs.Shireen. A.S., Horticulturist.

Preparation of extract

The leaves were air dried under shade. The dried leaves were powdered and passed through No 30 and stored in an airtight container for further uses. The collected, cleaned powder material of *Petroselinum crispum* Mill. was used for the extraction purpose. 220gm of powder was evenly packed in the soxhlet apparatus.

Petroleum ether extract of *Petroselinum crispum* mill.

The shade dried coarsely powdered leaves of *Petroselinum crispum* Mill. (400gm) was extracted with petroleum ether (60-80^{o}C), up to 72hrs. After completion of extraction, the solvent was removed by distillation. Dark green colour residue was obtained. The residue was then stored in a dessicator. The yield was found to be 2%w/w.

Ethanol extract of *Petroselinum crispum* mill

The marc left after Petroleum ether extraction was dried and then extracted with ethanol 95% (75-78^{o}C),up to 72hrs. After completion of extraction, the solvent was removed by distillation. Dark brownish green colour residue was obtained. The residue was then stored in a dessicator. The yield was found to be 32%w/w.

Aqueous extract of *Petroselinum crispum* mill

The marc left after ethanol extraction was dried and then extracted with chloroform water by cold maceration process. After completion of extraction, it was filtered and the solvent was removed by evaporation to dryness on a water bath. brown colour residue was obtained and it was stored in a desiccator. The yield was found to be 16%w/w.

Preliminary Phytochemical Investigation

The phytochemical constituents of various extracts of *Petroselinum crispum* Mill. were identified by chemical tests and the tests showed the presence of various phytochemical constituents like glycosides, saponins, carbohy drates, sterols, alkaloids, Flavonoids, tannins, proteins, triterpenoids.

Animals

Swiss albino mice (20-25gm) and Wister rats (150-200gm) of either sex and of approximate same age used in the present studies were procured from listed suppliers of Sri Venkateswara enterprises, Bangalore, India. The animals were fed with standard pellet diet (Hindustan lever Ltd. Bangalore) and water ad libitum. All the animals were housed in polypropylene cages. The animals were kept under alternate cycle of 12 hours of darkness and light. The animals were acclimatized to the laboratory condition for 1 week before starting the experiment. The animals were fasted for at least 12 hours before the onset of each activity. The experimental protocols were approved by Institutional Animal Ethics Committee (IAEC) CPCSEA Approval Number P.Col/56/2010/IAEC/VMCP after scrutinization. The animals received the drug treatment by oral gavage tube.

Toxicity Studies

Acute toxicity study was carried using Swiss albino mice with weight ranging (20-25 gm female) were taken for the experiment. The animals were made into a group of 3 each, dose of alcohol and aqueous attracts were given according to the body weight (mg/kg), starting dose of 5 mg/kg was given to the first individual animal, no death was occured, higher doses were given to next group of animal. (OECD GUIDELINE 423). There was no mortality found upto dose 2000mg/kg [12].

Pharmacological Screening

Anticoagulant activity

The rats were divided into 7 groups of six animals each. Group I served as a control, Group II rats were administered with Phytomena (10 mg/kg) orally, Group III rats were administered with standard drug of warfarin sodium (5 mg/kg) orally. Groups IV, V animals were administered with Ethanol extract of *Petroselinum crispum* mill (EEPCM) (200mg/kg, 400mg/kg respectively) orally. Groups VI, VII animals were administered with aqueous extract of *Petroselinum crispum* mill (AEPCM) (200mg/kg, 400mg/kg respectively) orally. After the end of the absorption time, they are anesthetized by intravenous injection of 60 mg/kg sodium pentobarbital. The retro-orbital bleeding method was employed to collect 1.8ml blood into a plastic syringe containing 0.2 ml 100 mM citrate buffer ph 4.5 (Behring werke, Marburg). The sample is immediately agitated and centrifuged in a plastic tube at 1500 g for 10 min. Plasma is transferred to another plastic tube and the coagulation test for the determination of thrombin time (TT), thromboplastin time (PT) and activated partial thromboplastin time (APTT) were performed within 3 h [13-17].

Statistical Analysis

The data were statistically analyzed by student's t-test and all the values were expressed as mean ± SEM. The data were also analyzed by one way ANOVA followed by Dunnet's t-test and values $p<0.05$ were considered significant.

Table 1. Anticoagulant Activity of the extracts of Leaves of *Petroselinum crispum* Mill. inVitro

Group no	Groups	Dose/ Route	aPTT (s)	PT (s)	TT (S)
I	Normal control	-----	38.5±0.6	12.83±0.83	19.33±1.33
II	phytomena	10 mg/kg(po)	27.33±2.04	10±0.36	14.16±0.98
III	Warfarin Na	5 mg/kg(po)	141.16±15.47***	20.50±1.33***	30.83±2.66***
IV	EEPCM	200 mg/kg(po)	92±3.32***	19.16±0.79***	25.16±0.83***
V	EEPCM	400 mg/kg(po)	111±2.17***	20±0.73***	25.33±0.55***
VI	AEPCM	200 mg/kg(po)	173.16±2.9***	23.16±1.08***	41±1.06***
VII	AEPCM	400 mg/kg(po)	176.83±2.78***	25.5±1.17***	44±1.12***

Values are expressed as mean ± S.E.M. (n=6),

- #, normal control group compare with phytomena treated group
- ### = p< 0.001, **=p< 0.01,*=p< 0.05
- *, compare with phytomena treated group
- ***= p< 0.001, ## = p<0.01, # = p< 0.05

Fig 1. Activated partial thromboplastin time (aPTT seconds) of plasma with different concentrations of *Petroselium crispum* mill. ethanol and aqueous leaf extracts and std drug of warfarin and negative control of phytomena of rats respondents (invitro)

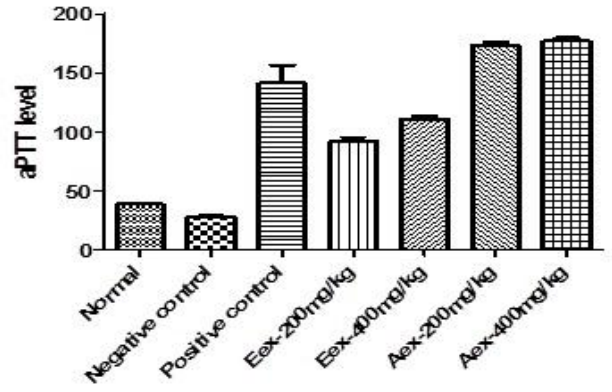


Fig 2. Prothrombin time (PT seconds) of plasma with different concentrations of *Petroselium crispum* mill. ethanol and aqueous leaf extracts and std drug of warfarin and negative control of phytomena of rats respondents (invitro)

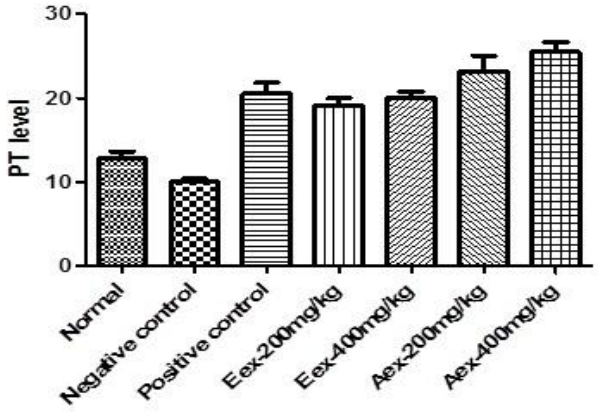
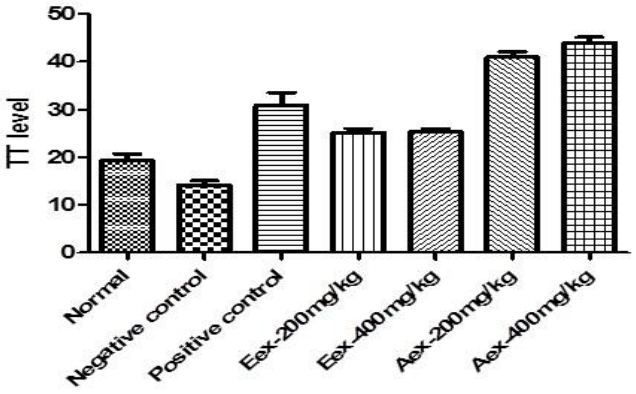


Fig 3. Thrombin time (TT seconds) of plasma with different concentrations of *Petroselium crispum* mill. ethanol and aqueous leaf extracts and std drug of warfarin and negative control of phytomena of rats respondents (invitro)



RESULTS AND DISCUSSION

The screening results of extracts for anticoagulant activities. The blood coagulation assays routinely used in screening procedures consisted of aPTT, PT and TT. In reference to Table no.5, all three coagulation parameters were significantly prolonged in plasma with ethanol and aqueous extracts (Group IV, V, VI & VII) compared to the normal control (Group I). There was a significant reduction in the level of aPTT, PT, TT activated time in phytomena (Group II) administered animals compared to the controls (Group 1).

Ethanol and aqueous extract of *Petroselinum crispum* mill leaves, whose aniicoagulant effectiveness is comparable to that of its commercial counterpart, warfarin, (Group III) showed a positive inhibition of clot formation with significant prolonged aPTT, PT, TT activated time In all the parameters studied, *Petroselinum crispum* mill ethanol and aqueous extracts at a doses of 200 mg/kg

showed a minor effect, whereas doses of 400 mg/kg showed significant effects. Aqueous extracts of *Petroselinum crispum* mill (200 mg/kg and 400 mg/kg) found to be the most effective than ethanol extracts of *Petroselinum crispum* mill (200 mg/kg and 400 mg/kg).

The extraction of medicinal plant materials involves the use of various solvents based on their ability to extract bioactive compounds of different solubility and polarities. However, disproportionate emphasis has been placed on the organic solvent extractants, thus overlooking the potential use of water. Cognizant to this aspect, extractions using both water and organic solvent i.e. ethanol were carried out in the present study. The net dried weight yields of the leaves extracted with aqueous and ethanol in this experiment showed that the highest yield was recovered from ethanol extraction method. The Aqueous extract that exhibited the most potent anticoagulant properties for all parameters tested in the in vitro screening. These results highlight the biochemical nature of the active compounds in *Petroselium crispum* mill leaf that might contribute to anticoagulation [18-20].

The extraction of medicinal plant materials involves the use of various solvents based on their ability to extract bioactive compounds of different solubility and polarities. However, disproportionate emphasis has been placed on the organic solvent extractants, thus overlooking the potential use of water. Cognizant to this aspect, extractions using both water and organic solvent i.e. ethanol were carried out in the present study. The net dried weight yields of the leaves extracted with aqueous and ethanol in this experiment showed that the highest yield was recovered from ethanol extraction method. The Aqueous extract that exhibited the most potent anticoagulant properties for all parameters tested in the in vitro screening. These results highlight the biochemical nature of the active compounds in *Petroselium crispum* mill leaf that might contribute to anticoagulation [21].

This method of extraction using water as a solvent has been highly lauded by the phytoscience community partly due to the effort to minimize the use of organic solvents. In addition to the nature of water as a universal solvent, it is an attractive option owing to its environmental friendly, non-flammable and nonhazardous attributes [22].

A growing fascination for natural anticoagulants discoveries stemming from the overwhelming consumer response seeking remedies devoid of unfavourable side effects has prompted the execution of this study. Consequently, *Petroselium crispum* mill leaf extract proved as a potential herbal-based anticoagulant candidate and demonstrated remarkable activities when subjected to a series of in vitro coagulation screening procedures. These measures are referred to as functional clot-based assays due to their ability to assess the formation of fibrin mesh as the ultimate end-product of the clotting process. Aqueous and ethanol extracts of *Petroselium crispum* mill leaf, whose anticoagulant effectiveness is comparable to that of its commercial counterpart, warfarin, showed a positive concentration-dependent inhibition of clot formation with significant prolonged a PTT.

An abnormally prolonged aPTT triggered by anticoagulant agents is associated with interference in the intrinsic coagulation pathway. This test is especially sensitive to the levels of factors VIII, IX, XI, and XII and to some extent can detect deficiencies of factors X, V and II. Conversely, the PT assay monitors the integrity of coagulation proteins, especially factor VII, in the extrinsic coagulation pathway. Thrombin time, as its name imply, measures the time consumed for thrombin-mediated fibrinogen conversion to fibrin clot. This investigation was therefore narrowed down to the intrinsic coagulation pathway. Immediate and timed incubation mixing studies were ensued to verify the underlying nature of aPTT prolongation by the *Petroselium crispum* mill extract.

CONCLUSION

The phytochemical and pharmacological studies on the extracts of leaves of the plant *Petroselinum crispum* Mill. was done. In the pharmacological studies, Ethanol and Aqueous extracts of leaves of *Petroselinum crispum* Mill showed significant Anticoagulant activity in dose dependent manner. It was found that Ethanol and Aqueous extracts of leaves of *Petroselinum crispum* Mill. were more effective in higher dose as compared to lower dose.

A present study reveals that the Ethanol and Aqueous extracts of leaves of *Petroselinum crispum* Mill. can be used as an effective Anticoagulant.

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REFERENCES

1. The Greatest Benefit to Mankind: A Medical History of Humanity from Antiquity to the Present. Harper Collins, ISBN 0-00-215173-1.
2. David Winston. American Extra Pharmacopoeia
3. Unschuld, Paul U. Huang Di Nei Jing Su Wen: Nature, Knowledge, Imagery in an Ancient Chinese Medical Text. Berkeley and Los Angeles: University of California Press, 2003.
4. Traditional Medicine. World Health Organization web site.
5. Farnsworth NR, Akerele O, Bingel AS, *et al.* Medicinal plants in therapy. *Bull World Health Organ*, 63, 1985, 965-981.
6. Beers M. Aging and the Cardiovascular System, The Merck Manual of Geriatrics. Merck & Co. Inc. 2006.
7. Guyton A and Hall J. Textbook of medical physiology. Philadelphia, 2000.
8. Johnson L. Essential medical physiology. Philadelphia, 1998.
9. Saladin K. Anatomy & physiology: the unity of form and function. Boston, McGraw-Hill, 2001.

10. Talley NS. Clinical examination: a guide to physical diagnosis. Sydney, MacLennan & Petty, 2001.
11. Connor O. Mediating vasoconstriction. *J. Vet. Pharmacol. Ther*, 20(4), 2001, 308–17.
12. Sagrada A, Fargeas MJ, Bueno L. Involvement of α_1 and α_2 adrenoceptors in the postlaparotomy intestinal motor disturbances in the rat, 2, 1987, 130.
13. Schmitz JM, Graham RM, Sagalowsky A, Pettinger WA. Renal α_1 and α_2 adrenergic receptors: biochemical and pharmacological correlations. *J. Pharmacol. Exp. Ther*, 219(2), 1981, 400–6.
14. Hylek EM, Evans-Molina C, Shea C, Henault LE, Regan S. Major hemorrhage and tolerability of warfarin in the first year of therapy among elderly patients with atrial fibrillation. *Circulation*, 115(21), 2007, 2689–96.
15. Adams J, Pepping J. Vitamin K in the treatment and prevention of osteoporosis and arterial calcification. *Am J Health Sys Pharm*, 62, 2005, 15,
16. New York Times. Drug Tied to China Had Contaminant, F.D.A, 2008.
17. New York Times. German Authorities Report Problems With Blood Thinner, 2008.
18. Blossom DB, *et al*. Outbreak of adverse reactions associated with contaminated heparin. *N Engl J Med*, 18, 2008, 359.
19. Di Nisio M, Middeldorp S, Büller HR. Direct thrombin inhibitors. N. Engl. Champe, Pamela C, 2006.
20. Harbourn JB. Phytochemical Methods-A Guide to modern Techniques of plant Analysis. Reprint, New York, 1976.
21. Kulkarani SJ. Handbook of Experimental Pharmacology, 3rd Edition, Vallabh Prakashan, 168-169.
Bounameaux H. The novel anticoagulants: entering a new era. Swiss. Med., Wkly, 2009.