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"PHYTOCHEMICAL EVALUATION AND PHARMACOLOGICAL SCREENING OF *CUPRESSUS SEMPERVIREN LINN. LEAVES EXTRACTS*"

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ABSTRACT

Herbal have a long traditional history or use of conventional medicine. The Cupressus Semperviren Linn. Garden plant belong to family cupressaaceae. The plant contain different chemical constituents like such as Glycosides, Essential oil, Phenols, Flavonoids etc. The main aim of the study was to extract the plant material by using pet-ether as a solvent by soxhelt extraction method. From the phytochemical analysis the plant was showed chemical constituents. The phytochemical constituent was isolation and separation by using thin layer chromatography and column chromatography. each chemical constituent were identified by using physical and chemical test. Isolated chemical constituent were subjected to further study that is it Spectral analysis like IR, NMR, And Mass spectroscopy the pet ether extact of plant was used for Pharmacological study such as Diuretic activity .the increase diuretic activity of plant was done by using LIPSCHITSZ test.

KEYWORDS: Cupressus Semperviren Linn., chromatographic analysis, Diuretic activity.

INTRODUCTION

Since 5,000 years the use of herbal medicine are documented. Herbal medicines are widely utilized as effective remedies for the prevention and treatment of multiple health conditions from centuries.[1] Many of medicinal plants have important role in the development of human culture. Aspirin like modern medicines are produced indirectly from medicinal plants. Many medicinal plant especially food crops have medicinal effects[2], example garlic. Many medicinal plants are resources of new drugs. The medicinal plant evaluated more than 250,000 flower plant species.[3] Medicinal plants helps to understand plant toxicity and also help to protect human and animals from natural poisons.[4] Cultivation and preservation of medicinal plants helpful to protect biological diversity for example metabolic engineering of plants. The medicinal effects of plants are due to metabolites especially secondary compounds produced by plant species.[5] Plant metabolites classified primary metabolites and secondary metabolites. Medicinal Phytotherapy is the use of plants or plant extracts. Long history and strong base for Ayurveda in india. Used traditional herbal medical system. Herbal plants used in preventing and treating of human diseases.[6] In thousand of year People have been using traditional medicine. The great importance of Natural products and traditional medicines. Ayurveda, Kampo, traditional Korean medicine, and Unani system of medicine used in some areas of world According to World Health Organization (WHO) 70% of the world's population proof on plants used primary health care and some 35,000 to 70,000 species medicament has been used, some 14-28% of the 250,000 plants species evaluated to occur around the world and in world-wide equivalent to 35-70% of all species Harbal medicine are not used in emergency condition like accident[7], serious illnesses as compared Modern medicine treats sudden and serious illnesses and accidents. Herbal medicine to treat serious trauma, such as a Heart attack. The crude extract of harbal plants may be used as medicaments and then Further processes the isolation and identification of the active ingredients and elucidation of active moiety Indian herbal drugs have been therapeutic uses are evaluated successfully and many of new medicinal plants are discovered.[8]

Classification

Plant Name- *Cupressus sempervirens Linn.*
Family –Cupressaceae
Synonym- Mediterranean cypress, Italian Cypress

Table no 1: Taxonomic Classification.

Kingdom	**Plantae**
Division	Pinophyta
Class	Pinopsida
Order	Pinales
Family	Cupressaceae
Genus	Cupressus

MATERIAL AND METHOD

Preparatin of Extract

The fresh leaves of cupress sempervarin L. were collected from local rregion of tasgoan Authentication of leaves was done by department of bonty Smt. Kasturbai walchand, sangli.

The collected leaves were washed with tap water and air dried under shead in house for 25-30 days after complete drying powder by mixture grinder to obtain fine powder.

The 100 gm of dried powder extracted with petroleum ether (40-60^0) in soxhelt extractor at temperature(40-60^0). The extraction was continued until the solvent in the thimble become a clear. After each extraction the solvent was recovered and extract was concentrated by using rotary evaporator at 60^0 temperature. After concentrated extract was stored in desiccators.

Phytochemical evaluation

The Pet ether extract (40-60^0) extract of cupressus semperviren. were used of qualitative chemical investigation to check various chemical constituent in the extract.

Test for glycoside +
Test for Tannin/ phenolic compound +
Test for Volatile oil +
Test for Flavonoids +

Phytsiochemical evaluation of Extract

Physiochemical evaluation of extract was performed by different method such as the total ash value and acid insoluble ash value, water soluble ash value and total moisture content. this method was used to remove the adulteration from the extract.

Isolation of pet ether extract of cupresus sempervarin

Isolation of cupreesus sempervarin extract carried out by using column chromtography Mobile phase used as petroleum ether (40-60^0):Ethyl acetate :Methanol (5:1:1) The isolated fraction were collected dried and used for further study isolated fraction identified by phytochemical TLC.

EXPERIMANTAL

In Vivo Diuretic activity

Invivo Diuretic activity

Determination of LD_{50} on rats using limit Test. As per OECD Guideline for testing of chemical (245) Principle of the limit test :- (acute toxicity)

Limit Test- The Limit test was sequential test that uses a maximum 5 animal. A test dose of 2000 or exceptional 5000mg/kg was used. The selection of a sequential test plan increase the statistical power.

Limit test at 2000mg/kg- The test dose was administered to one animal, if the animal dies, main test was conducted for determination of LD50. If animal survives additional four animals was sequentially used, so that total five animals was used.

Limit test at 5000mg/kg- The test dose was administered to one animal, if the animal dies, main test was conducted for determination of LD50. If animal survives, dose was administered to additional two animal.

LIPSCHITSZ Test model:- A method used for testing diuretic activity in rats has been described by LIPSCHTZ et. al (1943)

The test is based on water and sodium excretion in test animal an compared to rats treated with a high dose of urea.

The weighing 100-200gm Wistar albino rat of both sex were used for diuretic activity the animal were kept under controlled condition and fed with standerd pellets dites and water and libitum for one week.

Preparation of Extract for animal study

The crude Petroleum Ether extract of *cupressus semperviren L.* at 200mg/kg and 500mg/kg body weight were selected.

Evaluation

Animals will be divided into following groups, each group containing 6 animals and the treatment period of 1 days for whole study.

Group 1: Control (vehicle).
Group 2: Standard (Frusemide in single dose).
Group 3: Test group orally petroleum ether (40-60^0) Extract of *Cupressus Semperviren Linn.* leaves.(Lower dose)
Group 4: Test group orally petroleum ether(40-60^0) Extract of *Cupressus Semperviren Linn.* leaves. (Higher dose)

Biochemical Estimation

Total Urine Volume

Concentration of Sodium, Potassium and chloride ions

Na+ and K+ concentrations were measured using a flame photometer. The instrument was calibrated with standard solution containing different concentrations of Na+ and K+.

The Cl^- ion concentration was found by titration of samples with 0.02 N AgNO3 using 5 percent Potassium chromate solution as indicator. The results obtained were compared with the control test.

pH of urine.

Na^+ /k^+ Ratio.

RESULT AND DISSCUSION

Physical Test for Isolated Fraction

According to result the isolated fraction of plant extract was tested by using physical and chemical method

physical method was small test and chemical method was solubility of fraction and filter paper test.

Table no 2: Physical Test for Isolated Fraction.

Physical test		
Test	**Observation**	**Inference**
Small test	Characteristics Pungent Test	Essential oil present.

Chemical test		
Test	**Observation**	**Inference**
Solubility test	Soluble in water	Essential oil present.
Filter paper test	Oil spot disappear	Essential oil present.

Spectral Analysis

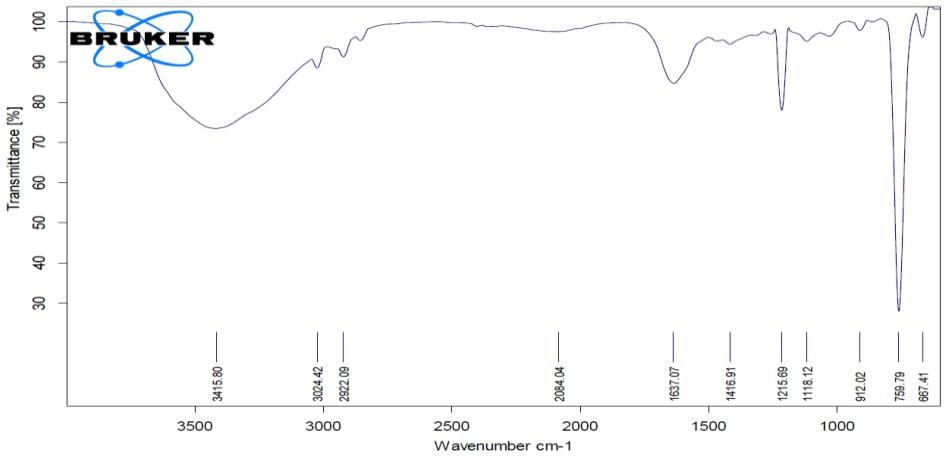

Fig 1: IR Spectra of Isolated Compound.

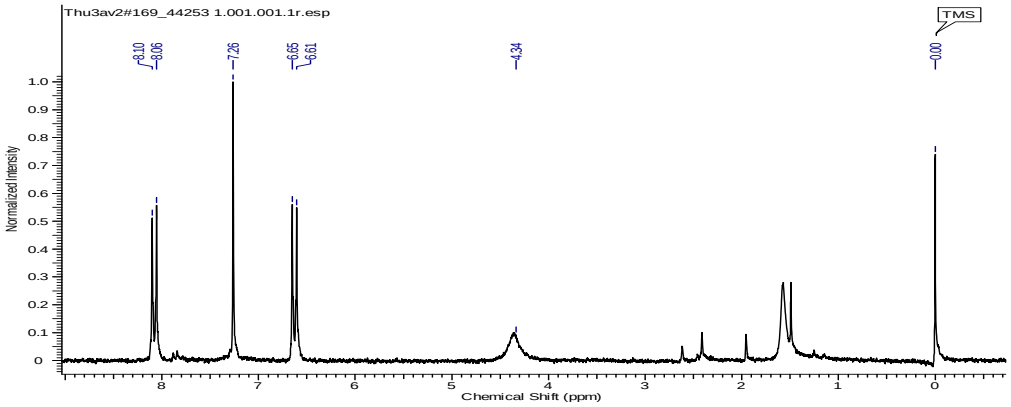

Fig 2: NMR spectroscopy isolated compound.

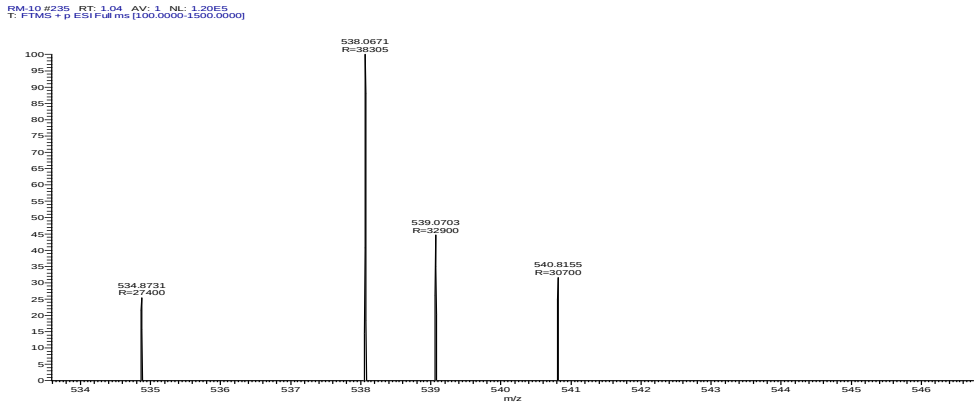

Fig 3: Mass spectra of isolated compound.

According to Spectral analysis the structure found cupressuflavon

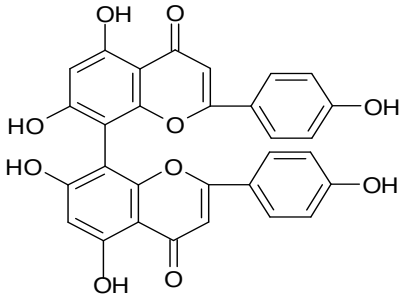

Fig 4: Cupressuflavon.

Isolated fraction was spectral analysis IR, NMR, MASS Spectroscopy The IR Spectra of compound shows absorption of bound of O-H str. at 3415.80, 3024.42, 2922. the C-H str. at 756.79, C=O str. at 1642.09 and C-O at 1215.69. All these absorption band shows presence of Flavonoid. The NMR spectra shows chemical shift at 6.65 shows presence of aromatic OH, also chemical shift at 8.18 shows presence of aromatic ring. The Mass spectra of obtained compound showed the molecular weight 538.46 and molecular ion peak 538.067. From these spectral analysis we were concluded that the resulting compound was Cupressuflavone which was Flavonoid in nature.

Pharmacological activity

Isolated Fraction further study pharmacological activity, Diuretic activity was perform by using LIPSCITSZ model. the test is based on water and sodium extraction in test animal and compaired to rat treated with high dose of urea According to activity the animal divided in four groups frist control group admimister water, second group administer std drug(13mg/kg), third (test-1 and test-2) and forth Group was administer extract(200,500mg/kg), in thses test the higher dose of extact collected volume of urea was increase compaird to control group Standerd drug.

Table 3: Effect of pet Ether extact of cupresus sempervarin.

Group	Dose (mg/kg)	Volume of urine (ml/5hr)	Volume of urine (ml/24hr
Control	-	2	2.7
Frusemide (std)	13mg/kg	7	8.5
Cupressus sempervirens(test1)	200mg/kg	4	5
Cupressus sempervirens(test2)	500mg/kg	6.8	8.4

The plant *Cupressus semperviren L.* leaves extract showed significant diuretic activity. Petroleum ether extract [Test 1 & Test 2] at dose 200mg/kg and 500mg/kg administered at rat the result showed that petroleum ether extract at dose 500mg/kg of *Cupressus semperviren L.* possess significant diuretic effect as compared to petroleum ether extract of *Cupressus semperviren L.* at dose 200mg/kg. The standerd drug frusemide at dose 13 mg/kg showed diuretic effect. The result obtained for both the standerd drug and petroleum ether extract treated rats were compared with control group Acording to result table-4 plant leaves extract fuether estimation of sodium and potassium lavel in urine by using colorimeter. The sodium level test 2 (2590.6****±1.55) extract was greater than test 1 extact (540.5****±0.5) also potassium level show significant effect the test 2 extact sample was greater potassium level (47.8****±0.15) compaird to test 2 (46.2****±0.55) extract sample and test 2 plant leaves extract showed significant Na+/ k+ ratio.

Table 4: Diuretic Effect of pet Ether extact of cupresus sempervarin.

Group	Urine pH	Sodium in Urine	Potassium in Urine	Na^+ / K^+ Ratio	Diuretic Value	Lipschitz Value
Control	7	430.2 ± 0.8	40.4±0.1	10.64	-----	-----
Frusemide (Standerd)	7.4	740.2****±1.01	50.8****±0.3	14.53	3.14	------
C.Semperviren L. Extract (test1)	6.8	540.5****±0.5	46.2****±0.55	11.69	1.8	0.58
*C.Sempervire L.*Extract (Test2)	7.2	590.6****±1.55	47.8****±0.15	12.35	3.11	1.68

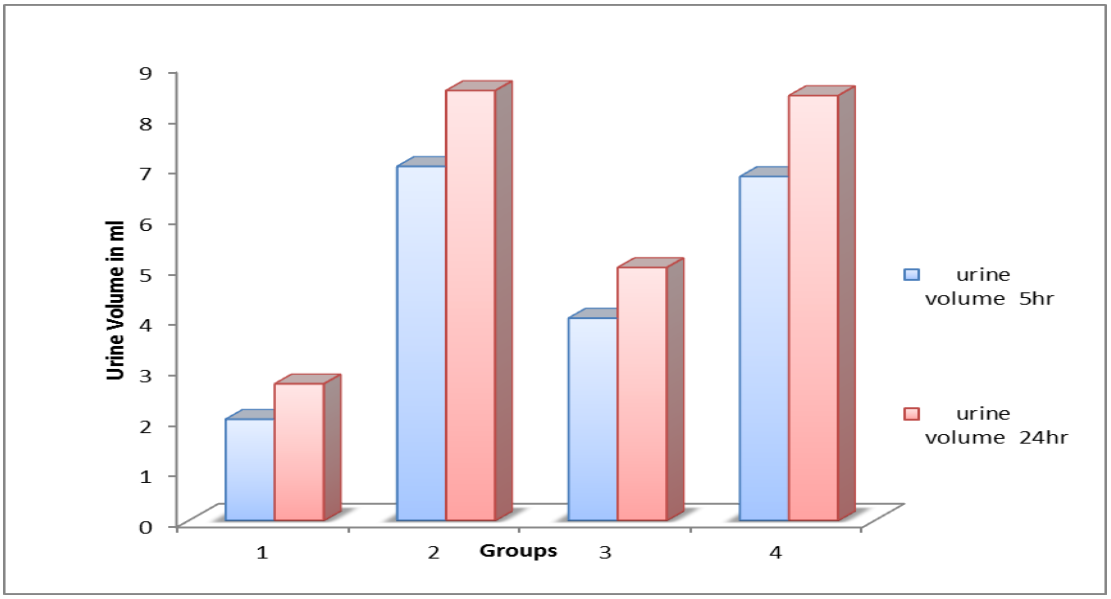

Fig no 6: Collected urine volume of Animal.

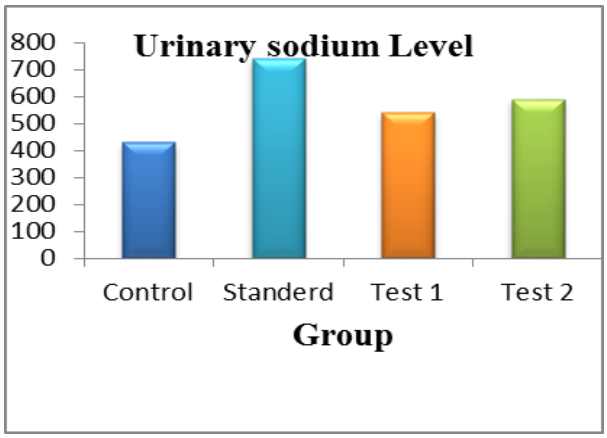

Fig 7: Effect of urinary sodium level.

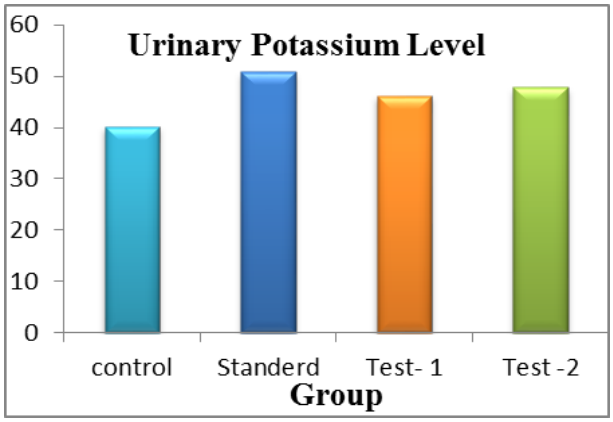

Fig 8: Effect of unirary potassium level.

CONCLUSION

From phytochemical evaluation it was found that presence of chemical evaluation and by thin layer and column chromatography these chemical constituents are separated and identified by spectral analysis from that it was concluded that the presence of Cupressuflavone which was flavonoids in nature.

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