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STUDY ON MEDICINAL IMPORTANT OF CELASTRUS PANICULATUS WILD

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ABSTRACT

The plants were recognised, and their authenticity was confirmed; additionally, a voucher specimen with the accession number 6890 was placed in the herbarium of the Department of Botany at the University of Calicut in India. Himedia in Mumbai, Merck in Mumbai, SRL in Mumbai, Sigma-Aldrich in Germany, and Qualigens in Mumbai were the places where I was able to get the chemicals that were utilised in the research. An endangered medicinal plant, Celastrus paiculatus Willd., which is a member of the family Celastraceae and is used in primary healthcare as well as in the production of a variety of herbal medication formulations (Rekha et al. 2005). Leprosy, leucoderma, skin illnesses, paralysis, depression, arthritis, asthma, and cancer are only few of the diseases that this treatment is effective against in the Ayurvedic medical system (Sharma et al. 2001, Rekha et al. 2005). In addition to its conventional applications, C. paniculatus has a variety of other uses that are common among indigenous peoples. The traditional applications of Jyotishmati are being revalidated by recent research. The careless harvesting of seeds and fruits could put Celastrus on the list of plants that are threatened with extinction.

KEYWORDS: *herbarium, medicinal, plants*

INTRODUCTION:

Herbal medicinal plants are used for the treatment of a wide variety of disorders, and it is not an exaggeration to say that the use of medicinal plants has been around for as long as people have been there to use them. The market for herbal medicine had a turnover of approximately US$ 30 billion in the year 2000 in the countries of the United States, Australia, and Canada. This figure had climbed by 5–15 percent by the turn of the century. The annual market for herbal medicine around the globe has already reached a staggering 60 billion dollars. Industrialized cultures have made discoveries that have led to the extraction of active ingredients from plants and the production of a number of medications and chemotherapeutic agents, both from the plants themselves and from rural herbal cures that have been traditionally employed.

The review of the relevant previous research for this study was carried out beginning in March 2018 and continuing up to the present day. "Celastrus paniculatus," "Malkangani," "Biological activities of Celastrus paniculatus," "Traditional uses of malkangni," and "Description of jyotishmati" were the search phrases that were utilised. The current investigation and search were carried out by consulting a wide variety of textbooks as well as periodicals that provide peer review and research papers. Science Direct, PubMed (which offers free access to Medline), and Google were the electronic databases that were utilised for this study. The more

in-depth research that went into creating the most recent review article on Celastrus paniculatus employed only previously published articles written in a variety of languages that were produced between 1970 and the present day. Cross-checks were also performed on the reference lists of individual publications.

Celastraceae

The Celastraceae family is widespread throughout the world's tropical and subtropical climates, including North Africa, South America, and various parts of East Asia, most notably China. The family Celastraceae is comprised of 1350 different species of herbs, vines, and small trees. It has 96 different genera. There are five different subfamilies that belong to the Celastraceae family. These subfamilies are called Celastroideae, Hippocrateoideae, Parnassioideae, and Salacioideae. Celastrus, Kokoona, Salacia, Gymnosporia, and Euonymus are all examples of genera that belong to the Celastraceae family. The leaves have a straightforward design that alternates. Flowers are both cymene and hermaphrodite at the same time. The endosperm of seeds is fleshy, and they have huge embryos and two sets of cotyledons. In the past thirty years, a variety of secondary metabolites have been isolated from Celastraceae. The primary components of these secondary metabolites are flavonoids, sesquiterpenoids, and phenyl alkyl amine.

Root bark

Alkaloids such as n-triacontanol and pristimerin were found in an extract of the root bark of Celastrus paniculatus made with petroleum ether. Also found were benzoic acid, an uncharacterized form of quinine, and a golden-yellow oil. In the outer bark of the root, we detected zeylaseral, zeylasterone, and celastrol in the form of qunione, methide, and phenolic triterpenoids.

Tannins are present in the ethanolic extract of Celastrus paniculatus, which was taken from the plant.

Ethnobotanical study

Since the beginning of time, man has made extensive use of the plants that make up the foundation of life on earth as a source of medicinal products. C. paniculatus is said to be used in the treatment of haemorrhoids, piles, gout, rheumatism, colds, dysentery, diarrhoea, leprosy, snake bites, and wounds in Himalayan traditional medicine (Agarwal, 2010). According to the documented history of Himachal Pradesh, the fruit juice of C. paniculatus is consumed as a cardio-tonic, while the seeds are consumed as an appetiser. On the scalp, a paste made of the fruit that has been combined with warm mustard oil is applied. The patient is then given the egg yolk that has had three drops of oil added into it. When taken orally with water, the powdered seeds (two to three grammes) are used to treat acidity and gas. The sun-dried fruit is next reduced to a powder and passed through a sieve. In order to eradicate intestinal worms, the patient must take orally 2-3 teaspoons of powder both in the morning and in the evening for a period of 4-5 days. Infected patches of skin are treated by oil application (Tiwari et al., 2010). In cases of headache, residents of the Haridwar area in the state of Uttaranchal have been known to use a poultice made from a paste made of C. paniculatus leaves and roots. In traditional Chinese medicine, pulverised roots are used to treat pneumonia (Chopra and Khanna, 2007). It is believed that the powdered root might be helpful in the treatment of malignant tumours by members of the Gond tribe who live in Uttar Pradesh (Parotta, 2001). Boils are treated by the indigenous people of the Achanakman-Amarkantak Biosphere Reserve (AABR) in Central India by applying a paste made from the root or the bark on the child's forehead.

Antifertility activity

The impact of the oily extract of the seeds of Celastrus paniculatus on the liver and testis of rats was investigated by Bidwai et al., 1990. They found that the extract had an antifertility effect. Vacuolization, cell depletion, and a stop in the development of spermatozoa were seen following intraperitoneal administration of 0.2 millilitres of oil extracted from Celastrus paniculatus for a period of thirty days.

The effectiveness of an ethanol extract of Celastrus paniculatus seed as an antifertility agent in male rats was reported by Singh and co-workers in 2018. The weight of the reproductive organs, the number of sperm, and their motility were all reduced in the rats after they were administered an oral dosage of 250 mg/kg for 45 days. The results of a biochemical analysis showed that the activity of the enzymes lactate dehydrogenase and gamma glutamyl transpeptidase in the testicles was up, whereas the activity of sorbitol dehydrogenase was lower.

Anti-inflammatory activity

An infection, an injury, or the presence of a foreign material might cause a tissue to react by going into an inflammatory state. In 1994, Ahmad and his colleagues investigated the potential anti-inflammatory action of a methanolic extract of the flowers of Celastrus paniculatus by subjecting mice to a hot water tail immersion test and subjecting rats to a carrageenan-induced edoema. According to the findings, flowers possess properties that make them both analgesic and antiinflammatory.

Nootropic activity

Gupta and Kumar, 2002 investigated the effects of aqueous, methanolic, chloroform, and petroleum ether extracts of Celastrus paniculatus seeds at the dose of 200 mg/kg for their potential nootropic properties in male Wistar rats. The researchers used a shuttle box, step through, step down, and elevated plus maze paradigms. Following a series of experiments, it was discovered that the rats' memories were only improved by the aqueous extract.

According to Bhanumathi and her colleagues' research from 2010, Celastrus paniculatus has also been shown to have nootropic properties. The raised plus maze and the passive avoidance test were the methodologies utilised for measuring nootropic activity. During the elevated plus maze procedure, the researchers administered doses of 350 and 1050 mg/kg of an aqueous extract of the seed of Celastrus paniculatus to the mice. During the passive avoidance test, the researchers administered dosages of 500 and 1500 mg/kg to the mice. Piracetam was administered at a dose of 100 mg/kg as the standard drug, while sodium nitrite was used to produce amnesia in the test subjects. According to the findings that were gathered, the Celastrus paniculatus seed extract improved memory capacity by suppressing the activity of the acetyl cholinesterase enzyme, which led to an increase in the amount of acetylcholine present in the brain.

Antifungal activity

The antifungal efficacy of Celastrus paniculatus mother plant leaves and in vitro grown clones was also examined by Sasidharan and Elyas, 2019 utilising chloroform and methanolic extracts against Phytophthora capsici and Rhizoctonia solani. On a medium consisting of dextrose agar, both the growth of fungi and their percentage were evaluated. In order to evaluate the cytotoxicity of extracts of Celastrus paniculatus leaves, a lethality assay with brine shrimp was carried out. The results of the experiments showed that the methanolic

extract of both the mother plant and the in vitro raised clones showed a hundred percent inhibition of Phytophthora capsici, whereas the chloroform extract of the mother plant showed very little activity, and the in vitro propagated plants showed only forty percent inhibition. Furthermore, the methanolic extract showed maximum activity against solani, whereas the chloroform extract showed 77.77 percent and 86.66 percent respectively.

OBJECTIVE

1. The elicitation of total phenolics in the callus cell suspension culture by using a variety of elicitors such as jasmonic acid, salicylic acid, and copper sulphate.

2. Using HPLC, determine the presence of celastrol in the mother plant as well as the in vitro produced clones of C. panicultus, and determine the amount of celastrol present.

MATERIALS AND METHODS

Himedia in Mumbai, Merck in Mumbai, SRL in Mumbai, Sigma-Aldrich in Germany, and Qualigens in Mumbai were the places where I was able to get the chemicals that were utilised in the research. The analytical grade of the compounds was what was used. There was no element of predictability in the experimental design.

The newly gathered leaves were given a quick wash in running tap water, and then they were given a final rinsing in distilled water. After that, the leaves were pulverised after being dried in the shade. In preparation for continued use, the powder was sealed away in airtight containers.

Physiochemical qualities and characteristics.

Following Mukherjee's methodology, a number of physicochemical properties were analysed and characterised (2002). The leaf powder was analysed for a number of different physiochemical parameters, including loss on drying, ash value, total ash value, acid-insoluble ash value, pH, aqueous extractive value, and the extractive value of a number of different organic solvents, including petroleum ether, chloroform, ethyl acetate, and methanol. All of these measurements were taken before and after the powder was dried.

An examination of fluorescence in leaf extracts.

The plant's young leaves were picked off and taken away. After being dried in the shade for two to three weeks, the leaves were then pulverised into a coarse powder. The extract was prepared using the method of extraction using a single solvent. In a conical flask containing 250 millilitres of liquid, ten grammes of the powder were combined with one hundred millilitres each of petroleum ether, chloroform, ethyl acetate, and methanol. The mixture was left at room temperature for twenty-four hours. After passing the suspension through Whatman's filter paper, the contents of the suspension were collected in big Petri plates. The temperature of the water bath was kept at 40 0.2 degrees Celsius during the drying process, which took thirty minutes. The dried extracts were removed by scraping them out with scalpels and were collected in separate vials that had been pre-weighed before the analysis continued.

Screening for potential phytochemicals at an early stage.

In order to analyse the pharmacological properties of the plant material, it was utilised in its unprocessed state while also being subjected to extraction using appropriate solvents in order to obtain the needed components. Because herbal medicines include such a large number of different chemical compounds, it is essential to separate out the active components that are responsible for the therapeutic benefits. Before determining the extracts' biological activity, it is necessary, therefore, to determine what the nature of the extracts is (Gupta et al.2003). Following the usual methods of Harborne (1973), Trease et al. (1989), Sofowara (1993), and Khandelwal (2008), the preliminary qualitative analysis was carried out to identify the bioconstituents. These approaches are explained in more detail below.

Conduct analyses to look for sugars and glycosides.:

After dissolving each extract separately in 4 cc of distilled water, the resulting mixture was filtered to remove any impurities. In order to determine whether or not the filtrate included carbohydrates and glycosides, the following tests were carried out on it.

The filtrate was subjected to the Molisch test, which involved treating it with 2–3 drops of 1 percent alcoholic alpha- naphthol and adding 2 ml of concentrated hydrogen sulphide solution along the edges of the test tube. The presence of carbohydrates can be determined by the brown ring that forms at the boundary between two liquids when they are mixed.

The filtrate was subjected to the Fehling's test, in which it was heated on a water bath after being treated with 1 ml of Fehling's solution. A precipitate with a reddish colour was observed, which indicates the presence of carbohydrates.

Another portion of the extracts was hydrolyzed with diluted hydrochloric acid for a few hours on a water bath, and the resulting hydrolysate was put through the following assays to determine whether or not glycosides were present.

The hydrolysate from the previous step was subjected to chloroform treatment, and the resulting chloroform layer was subsequently separated. This was followed by the addition of an equal volume of a diluted ammonia solution. The presence of glycosides can be identified by the pink coloration of the ammonia layer.

Identification of oils that are fixed:

The filter paper test consisted of pressing a little sample of extract individually between two pieces of filter paper. The appearance of a stain that looks like oil on the paper is evidence that there are oils that are fixed there.

Proteins as well as free amino acids can be identified:

Following the dissolution of a small quantity of extracts in a few millilitres of distilled water, the samples were put through the following test.

The Biuret test consisted of adding 2 millilitres of a solution containing 5 percent NaOH and 1 millilitre of CuSO4 to the extracts that had previously been produced. The presence of proteins as well as free amino acids is shown by the colour violet.

Chromatography with a Thin Layer

Using thin layer chromatography (TLC) on analytical plates over silica gel, we were able to separate the secondary metabolites that were found in the leaf extracts and conduct an analysis of those compounds (Ahumada et al.1991 and Ansari 2001). Plates were made by hand, then air dried, and then activated in an oven at 1100 degrees Celsius for one hour prior to use. At a concentration of 1 mg ml-1, the plant extracts that were discussed previously were each given a spot size of 10 l and placed on TLC plates. In order to achieve higher resolution and more consistent separations, a variety of solvent systems and combinations were put through their paces. Ethyl acetate, Methanol, and Water (1:2:1), Toluene, and Ethyl acetate (3:7), and Methanol, Chloroform (1:1) are some of the more common ones (9:1). Toluene: ethyl acetate (3:7) displayed the best findings, and as a result, this combination was chosen for the purpose of conducting additional research

Inside of a chamber that was saturated with solvent, the spotted TLC plates were allowed to develop. The developed plates were then air dried before being visualised in visible and ultra violet light (254 nm and 365 nm), as well as by spraying with various reagents such as the Dragendroff reagent for alkaloids, the Fast blue salt reagent for detection of the phenolic group, ninhydrin for aminoacids and biogenic amines, vanillin phosphoric acid for detection of terpenoids, and anisaldehyde- In order to separate the phenolic components, the same solvent system was used, and after that, post-chromatographic derivatization was carried out using an ethanolic solution of 2 percent FeCl3. Marks were made on the colourful spots that were present on the silica gel plates, and the distances between them were measured so that the chromatographic behaviour of the sample components could be expressed as their retention factor, or Rf value. The following equation was used to get the Rf value of each active ingredient in each extract:,

$$\text{Rf value} = \frac{\text{Distance from base line travelled by the solute}}{\text{Distance from the base line travelled by solvent (Solvent front)}}$$

RESULTS

The colour characteristics of the plant powder as such as well as after treating them with various chemical reagents were observed in day light as well as under UV radiation at 254 nm for short wavelengths and 365 nm for long wavelengths. This was done to compare the colour characteristics of the plant powder before and after the treatment. The information regarding the colour formation with regard to the specific reagents was documented and may be seen in table 4.1. This distinctive colour reaction can help determine the quality and purity of the leaf powder by providing additional information. In response to a number of different chemical reagents, the leaf powder of both in vitro propagated and the mother plant of C. paniculatus demonstrated the same outcome.

Table 4.1 Utilizing a variety of chemicals, a fluorescence study was performed on the leaf powder of both the mother plant and in vitro grown clones of C. paniculatus.

Sl No.	Particular of Treatment	Mother plant			*In vitro* propagated plant		
		Under visible light	Under UV light (254 nm)	Under UV light (365 nm)	Under visible light	Under UV light (254 nm)	Under UV light (365 nm)
1	Powder alone	Green	Green	Green	Green	Green	Green
2	Powder + 1N aqueous NaOH	Green	Green	Green	Green	Green	Green
3	Powder+1N alcoholic NaOH	Green	Light green	Light green	Green	Light green	Light green
4	Powder + 1N HCl	Dark green	Orange	Orange	Dark green	Orange	Orange
5	Powder + NH_3	Green	Yellow	Green	Green	Yellow	Green
6	Powder + Iodine	Light green	Pink	Pink	Light green	Pink	Pink
7	Powder + 5% $FeCl_3$	Green	Orange	Light green	Green	Orange	Light green
8	Powder + acetic acid	Green	Green	Green	Green	Green	Green
9	Powder + HNO_3	Brown	Yellow	Yellow	Brown	Yellow	Yellow
10	Powder + $H2SO_4$	Black	Dark black	Dark black	Black	Dark black	Dark black

physiochemical qualities and characteristics

Measurements were taken of a variety of physiochemical parameters in the leaf extracts of both in vitro grown and the mother plant of C. paniculatus. A number of different values, including total ash value, acid soluble ash value, water soluble ash value, and extractive value, were calculated. The findings show that the loss on drying in the sample was 6.26 ±0.30 percent w/w for the mother plant and 6.12 ± 0.32 percent w/w for the in vitro propagated plant. This indicates that the value of the sample's moisture content is lower than the value of the moisture content in the mother plant. Inorganic material was present in the sample based on its total ash value, which came in at 14.32 ±0.13 percent w/w for the mother plant and 14.24 ±0.11 percent w/w for the plant that was propagated in vitro. The fact that the aqueous extractive value is greater (11.53 ±0.42 percent w/w for the mother plant and 11.53 ± 0.41 percent w/w for the in vitro propagated plant.) is an indication that the sample medication is more soluble in water. The aqueous extract contains relatively little fat or resin, but it does have a significant concentration of other components, such as tannin, amino acid, sugar, glycosides, and carbohydrates. The leaf has a somewhat acidic nature, as indicated by the pH of a solution containing 5 percent water. The yield of the solvent extracts as a percentage might range anywhere from 3.33 to 13.26, depending on the solvent. The amount of chloroform and petroleum ether extract that could be harvested from the plant being investigated was insufficient. Because the leaves contain a higher concentration of methanol-soluble elements, the methanolic extract had a comparatively very high extractive value. This can be explained by the fact that methanol dissolves the constituents more easily .

Discussion

In the current investigation, callus cell suspension cultures were initiated, maintained, and analysed for the generation of total phenolics. These cultures were derived from C. paniculatus. Because the friability of the callus has a significant impact on the establishment of the cell suspension culture derived from the callus, creamy friable callus that was generated from MS medium that had been treated with 2,4-D (1.5 mgl-1) and

NAA (1 mgl-1) was utilised in this study. Bhojwani and Razdan (1990) found that the level of friability of the callus tissue increased when it was maintained on a semisolid media for two or three passages. This was the case when there was an increase in the degree of friability. For the purposes of this study, full strength MS liquid medium was utilised for the purpose of beginning cell suspension cultures derived from C. paniculatus. According to Nagella et al. (2011), full strength MS medium was utilised for the Gymnema sylvestre suspension culture experiments. [Citation needed] According to the available research, the growth kinetics of plant cells dictate which growth and production media should be used for cell suspension cultures in order to achieve optimal results (Coste et al.2011). During the incubation period, a gradual increase in the biomass production of cell suspension cultures was observed, and this was analysed by measuring the fresh weight and the dry weight. During the incubation period, a gradual increase in the biomass production of cell suspension cultures was observed.

Both the fresh weight and the dried weight of the callus cell suspension culture were considered in the research on the growth kinetics of the culture. The cells entered the lag phase after three days of incubation in liquid MS media. At this point, the cells were not actively dividing. After this comes the exponential phase, which lasts between 6 and 20 days. The decline phase began between the ages of 20 and 30 days, and it was characterised by a slow but steady loss of both fresh and dry weight. This can be a result of the depletion of nutrients and the deficiency of oxygen in the medium, and it ultimately leads to the death of the cells. According to the results of the study, the profile of secondary metabolites found in cell suspension cultures is comparable to that seen in whole plants. The methanolic extracts were the ones that revealed the greatest diversity of secondary metabolites compared to the other solvent extracts. The results of the phytochemical analysis performed on seed extracts of C. paniculatus by Venkataramaiah et al. are consistent with this finding (2011).

CONCLUSION

In the course of research on in vitro propagation of C. paniculatus, a highly reproducible methodology was developed for the induction of shoots, the multiplication of shoots in vitro, the in vitro roots of explants, and the induction of calluses from a variety of explant sources. On MS media that had been added with 1.5 mg/l of BAP, it was discovered that the frequency of shoot induction increased.The percentage of people that responded from the culture was higher than fifty percent. After a period of four to five weeks, a number of shoots along with a well-developed root system were created. The disc culture method on potato dextrose agar medium was used for the confirmation of the pathological screening that was performed to determine if in vitro grown. Using a variety of different phytochemical methods, a comparison was made between the chemical contents of in vitro produced clones of C.panicultus and the mother plant. After drying, the leaves were processed using a single solvent extraction method, which resulted in the production of extracts of four different solvents, including petroleum ether, chloroform, ethyl acetate, and methanol. The phytochemical study revealed the presence of several different types of chemicals, including alkaloids, flavanoids, steroids, saponins, tannins, and phenolic compounds. The findings of the TLC analysis demonstrated that the extract contained a significant amount of phenolic chemicals. The fact that the HPTLC fingerprint profiles of the in vitro produced clones and the mother plant were exactly the same indicates that they have a high degree of chemical similarity.

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