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EXPERIMENTAL INVESTIGATION OF PHARMACOLOGICAL SCREENING OF CATARCT USING SOME MEDICINAL PLANT EXTRACT

Dr. Subhashini Sharma*

Associate Professor Dept of Chemistry Mmh College Ghaziabad U.P. India.

***Corresponding Author: Dr. Subhashini Sharma**
Associate Professor Dept of Chemistry Mmh College Ghaziabad U.P. India.

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ABSTRACT
It is the goal of this study to identify and quantify the phytochemical constituents, as well as the level of antioxidant, cytotoxic, anti-microbial and in vivo analgesic, anti-inflammatory, and anti-diarrheal activity of the root ether (methanol) and aqueous (aqueous) extracts of the roots. Most extracts, however, included flavonoids and phenolic substances. Experimental hydrogen peroxide-induced cataractogenesis seems to be greatly slowed by methanolic extract of Acorus calamus, isolated chemical (asarone), and standard medicine (Catlon and Tobramycin), according to the findings.

KEYWORDS: Isolated, Acorus, Phytochemicals, Flavonoids, Analgesic.

INTRODCUTION
For many years, plants have served as the foundation for the creation of contemporary pharmaceuticals, which have been used to treat a wide range of disorders across the globe. Herbs have long been utilised to enhance health and well-being in traditional communities, whether in Africa or elsewhere. Numerous traditional medicines may be found at a fraction of the cost of contemporary alternatives in rural regions. To manufacture microbicides and other insecticides and pharmaceuticals, plants often develop a large number of secondary metabolites. Antimicrobial and antioxidative characteristics of medicinal plants are becoming more popular, both in business and in scientific study. In addition to flavanoids, terpenoids, carotenoids, coumarone, and curcumines, these active phytochemicals contribute to the characteristics. Emetine, quinine, berberine, tannins, terpenoids, alkaloids, and flavonoids, all derived from plants, have long been recognised as potent weapons in the battle against infectious diseases. About 80% of the world's population depends on traditional medicines for their basic healthcare requirements. More than 50,000 of the world's 422,000 blooming plants have been identified as having therapeutic use.

In spite of recent technical advances in eye surgery, cataract remains the leading cause of global blindness, despite these advances. Around 32 million individuals were blind in 2010 and 191 million had impaired eyesight. Cataract was the cause of blindness for one out of every three persons (Khairallah et al., 2015). 90 million people worldwide will be blind by 2020, according to the World Health Organization (WHO) (Khairallah et al., 2015; Taylor, 2016). Human resources, infrastructural development, and disease management are all part of the approach to combat this problem. The latter is determined by the individual disease's features. In Europe, the prevalence of cataracts rises from 5% for individuals aged 52–62 to 64% for those aged 70 and above (Prokofyeva et al., 2013). Age is a risk factor for cataracts that cannot be changed, thus the rapid ageing of the population is a cause for concern. It is critical to identify cataract risk factors that may be changed, since this will aid in the development of preventative interventions.

Traditional treatments are almost often based on crude plant extracts that include several chemical ingredients, ranging in intensity from very potent to extremely weak. Instead, conventional medicine makes heavy use of single, chemically well-characterized active substances that have been shown to have specific effects on already well-established biological targets. Many of these drugs have tight dosage windows between therapeutic and harmful effects, making them very strong. The bioavailability of orthodox medications is ensured by preparing them in precise dosages. Traditional medications derived from chemicals in higher plants include analgesics, antimalarials, antitumors, and asthma remedies.

Literature Review
Marina Chemerovski-Glikman (2018) Lens crystallin proteins aggregation is what causes cataracts to form, which is the most common reason for visual loss. Only

surgical removal is now available as a treatment option. Recent discoveries have sparked a resurgence in the search for non-surgical therapy options. New procedures, on the other hand, have not yet been able to consistently and completely remove lenses. The first ex vivo technique for the screening of medication candidates that minimise the aggregation of human lenticular protein was established in this laboratory. Restoring virtually entire clarity to phacoemulsified cataracts ex vivo was made possible thanks to this test, which identified two key molecules. Cataract microparticles may be reduced and their amyloid-like characteristics altered by both substances, according to investigations on their mechanisms of action. rosmarinic acid has been shown to slow the development of cataracts and lower the degree of lens opacification in laboratory animals. As a result, the ex vivo experiment might serve as a starting point for further research into new therapeutic compounds for the treatment of cataracts.

Tewari D (2019) If any lens opacity or loss of transparency is present in your eyes, you have cataracts and are at risk of becoming blind. Vision impairment, colour disruption, and glare are some of the most prevalent symptoms of cataracts. As we age, our eyes become more susceptible to the effects of oxidative stress. Although phacoemulsification and intraocular lens implantation is the most efficient way to cure cataracts, there is a risk of major complications and permanent loss of vision. Anticataract drugs may be developed from natural secondary metabolites that are antioxidants or anti-inflammatory. To the best of our knowledge, this is the most comprehensive study to date on the topic of plant-based cataract treatments.

Pasumarthy N.V. Gopal (2016) The eye is one of the body's most sensitive organs, and it is constantly bombarded with a variety of harmful substances. Conjunctivitis, cataract, ocular allergies, glaucoma, and inflammation are among the most common eye diseases. Because of the negative effects of allopathic medications, herbal medicines have become the primary therapy for ocular illnesses in recent years. In both developed and developing nations, cataract is the most common cause of blindness, accounting for 50% of all cases. Cataract surgery is a common and very inexpensive method of treating this visual disease. By summarising relevant information, this review aids in the non-surgical treatment of cataracts using various plant materials.

Zelalem Abdisa (2020) Clematis hirsuta, a member of the Ranunculaceae family, may be found growing high in the Ethiopian mountains. Clematis hirsuta's leaves and roots are historically used in Ethiopia to cure a variety of ailments. An investigation of the phytochemical components, antibacterial and antioxidant properties of Clematis hirsuta root extract is the goal of this work. The powdered air-dried plant sample was extracted using n-hexane, chloroform, and methanol in that order. A rotary evaporator was used to concentrate the filtrates after they had been filtered. Standard techniques were used to conduct a preliminary screening of the dried extract for the presence of alkaloid, saponin and tannins as well as flavonoids and carbohydrates. The disc diffusion technique was used to test the crude extract's antibacterial activity.

Staphylococcus aureus, E. coli, P. aeruginosa, and Salmonella thyphi were all inhibited by Clematis hirsuta root methanol extract, whereas chloroform extract was inhibited by P. aeruginosa and Staphylococcus aureus. The DPPH test technique was used to screen the methanol crude extract for antioxidant activity. Ascorbic acid (95.8 percent) inhibited DPPH at a concentration of 2 mg/mL in the same amount of methanol with an inhibition percentage of 98.2 percent. The roots of Clematis hirsuta were found to have phytochemicals with antibacterial and antioxidant properties, indicating that they might be useful in the quest for natural antibiotics and antioxidants derived from plants.

Pharmacological strategies for prevention of cataract

Aldose reductase inhibitors: The goal of ARIs is to obstruct the glucose metabolism pathways that lead to diabetic vascular disease. It's now well-established that they help keep diabetic cataracts at bay in animals. Aldose reductase has been discovered to be inhibited by several natural and manmade substances. To stop polyol formation, these so-called ARI attach to aldose reductase. Due to the slowness of the aldose reductase, the justification for sorbitol-lowering medicines has diminished with time. Furthermore, sorbitol does not accumulate in adult human lenses incubated in high glucose medium.

Non-steroidal anti-inflammatory drugs: A new class of anticataractic medications has emerged: non-steroidal anti-inflammatory drugs (NSAIDs). Studies on aspirin usage in individuals with rheumatoid arthritis and diabetes provided the first hint that NSAIDs may be used as preventive anticataract medicines. There have since been reports of NSAIDs with a wide range of chemical configurations affecting experimental animals. An investigation of aspirin, sulindac and naproxen eye drops for their anticataract properties was also carried out, with no detrimental side effects seen even after long-term use of these eye drops.

Agents which act on glutathione: Deactivation and neutralisation of free radicals are two of glutathione's most critical jobs. Cysteine, glutamic acid, and glycine are the three amino acids that make up GSH, and the lens synthesises it in two ATP-dependent processes. One of the many ways in which GSH or the lack thereof may impact the lens's opacity.

Morphology

This plant has long, slender leaves that radiate out from a pinkish base and may reach 1.5 metres in length. Curved spadix, or flower stalk, with little yellowish-green to brown flowers on it. It produces green, angular berries with one to three seeds. In terms of morphology, the seeds are elongated. There are various nodes and internodes on the plant's rhizomes, which may vary in shape from cylindrical to flat. The rhizomes have a strong, distinctive perfume and flavour that is bitter. There are small cortical and vast stellar zones delineated on the transversely sliced surface of the rhizome with a pinkish tint. Cells in the epidermis are drastically enlarged, with thicker walls, when seen microscopically. The cortical area is composed of chains of thin-walled parenchymatous cells with vast intercellular gaps, sheathed collateral vascular bundles, and patches of fibres.

Cultivation

Rhizomes are used to spread the plant. The emerging rhizomes come from mature plants that are still growing strong. For 5 minutes, a 0.3 percent solution of brassical should be applied to propagates before to planting. Using furrows at a depth of 6cm, the plants are planted 30cm apart and 35cm apart in rows. Rhizomes need little watering after planting in order to grow uniformly and well.

Chemical constituents

There have been several reports of chemical components in the rhizome of Acorus calamus. Researchers have examined the chemical composition of the oil from Acorus rhizomes. The oil includes various concentrations of -asarone(a), -asarone, asarone, calamine, calamemenol, calamenone(b), -pinene(c), -pinene(d), camphene, eugenyl acetate, eugenol(e), isoeugenol(f), methyl eugenol(g Palmitic acid and its ester, heptylic acid, an ester of butyric acid, and acoramone are among the fatty acids found in the oil (k). These compounds are found in the rhizome extract, which is 50 percent ethanolic. Sweet flag oil yielded two sesquiterpenoids ketones, calamusenone (l) and its isomer, both of the guaiane type. Acorus also yielded the sesquiterpenoids shybunone(m), isoshybunone(n), isocalamendiol, dehydroxycalamendiol, and epishyobunone, in addition to calamenone. preisocalamendiol, a germacrone molecule, and acorone were produced by thermal isomerization of shybunone, an elememe(o) type sesquiterpene (p).

Extraction of Acorus calamus (roots)

In order to get an aqueous extract, we macerated 500 grammes of dried root powder in 60°-80°C petroleum ether, methanol, and distilled water in a Soxhlet apparatus (Perfit, India). This was followed by a series of extractions that were filtered and the resulting filtrate evaporated under decreased pressure in an autoclave (Perfit, India) heated to 50° C1. After evaporation, a desiccator was used to hold the crude extract. The residual residue of the root was removed and the extract was weighed after extraction with different solvents. Table 1 lists the physical characteristics and % yield of each extract.

Table 1 Physical nature and percentage yield of various root extracts of Acorus calamus.

Isolation of compound from Acorus calamus roots

Slurry was made from the methanolic extract green residue soaked in a small quantity of chloroform in a round bottom flask and absorbed over a silica gel mesh size 60-120 that had been used in the extraction process before. A rotatory evaporator was used to dry the slurry at a lower pressure (Perfit India). To get the methanolic extract, the dried silica gel slurry was put into the column (302 cm) that had previously been packed with chloroform-soaked silica gel slurry (60-12 mesh size). Different solvents were used in ascending order of polarity for elution. The conical flasks in which the fractions were gathered were labelled. The homogeneity of distinct fractions was examined using thin layer chromatography on the fractions. There was a pooling and concentration of Rf values. It was possible to isolate a single chemical by isolating the fractions 12–18 that had the same Rf (0.85) (AC-1). FTIR, 1H NMR, and mass spectroscopy were all performed on an isolated chemical (AC 1). Figure 1 shows the FTIR spectra of the isolated chemical (AC 1) from the roots of Acorus calamus (roots).

Fig. 1 FTIR spectra of the isolated compound of Acorus calamus

As can be seen in the FTIR spectrum, bands at 2929.7.2 cm-1 suggest that C-H stretching occurs in alkanes. C=C stretching of the aromatic ring was detected at 1645.4 cm-1 and 1510.5 cm-1. At 1053.5 cm-1, ether (C-O stretching) exhibited a strong peak. The tetra-substituted benzene ring was found to have a distinct peak at 802 cm-1. The FTIR spectra of Acorus calamus, an isolated substance, are shown in the table 2.

Table 2 Interpretation of FTIR spectra of the isolated compound of Acorus calamus

Anticataract activity

Acorus calamus, Vitex negundo, and Butea frondosa have been historically utilised in the treatment of eye illnesses and disorders as a topical preparation. 1 Cataract has been the leading cause of blindness since the dawn of civilization. Cataract aetiology has been linked to oxidative stress. 2 Ayurveda mentions the use of Acorus calamus, Vitex negundo, and Butea frondosa in the treatment of eye problems. The current research examined the anticataract potential of Acorus calamus, Vitex negundo, and Butea frondosa. In DMEM, all of the lenses were clear. Lenses, on the other hand, acquired dense opacity after 52 hours of incubation with hydrogen peroxide. When exposed to hydrogen peroxide (H2O2), goat lenses were shown to be significantly protected by the methanolic extract of Acorus calamus (Acorus calamus extract). It was shown that 0.25 mg/ml of Acorus calamus extract at 0.25 mg/ml avoided opacity for 21 to 44 hours compared to the control, which turned opaque in 17 hours, while conventional medicine Catlon + Tobramycin (Catlon) prevented opacity for 46 hours. Table 3 shows the anticataract efficacy of Acorus calamus extract and an isolated chemical against hydrogen peroxide-induced cataract.

Table 3 Anticataract activity of the extract of Acorus calamus and isolated compound against hydrogen peroxide induced cataract.

Each values represents mean ± S.E. n=6;

CONCLUSION

The purpose of this research was to investigate the anticataract properties of Acorus calamus extract (roots), An exhaustive review of the literature found that these plants' blooms and seeds had already spawned an abundance of research. Leaves and roots, on the other hand, have received much less study. As a result, we set out to investigate the ocular activities of leaves and roots, which are a particularly rich source of phytoconstituents. Inflammation, intestinal worm infection, urinary problems, piles, natural colourants, chronic dysentery, backache, aphrodisiac, and skin ailments have all been treated using the seeds, flowers, bark, and gum of these plants. According to ayurveda, it has ocular action. Because this activity has never been documented scientifically, the current research was carried out to assess its anticataractic potential and to back up the traditional assertions. We used ethanol and methanol in addition to water to extract the Acorus cauliflorum roots. Reported to be 6.58 percent W/W, the oil-ether extract yield was found to be 6. This light yellow semisolid was the extract. It was discovered that the root had a methanolic extract yield of 25.58 percent w/w. Deep crimson semisolids were formed from the extract. In root, aqueous extracts yielded a 20% w/w yield. An amber-brown semisolid was the result of the extraction. In the roots of Acorus calamus, all extracts were found to be semisolid in consistency.

Future Directions

An exhaustive study of A. calamus' ethnomedicinal usage and commercial formulations as well as its chemical ingredients and the pharmacological properties of crude, n-hexane and Ethyl Acetate extracts, together with pure compounds and clinical studies, is included in this paper. Studying extracts and compounds of A. calamus indicated anti-diabetic potentials as well as a variety of unique signalling pathways. Some of the chemical ingredients (such as -asarone, -asarone, and eugenol) have been studied for their biological potential and action mechanisms. Other chemicals, on the other hand, need scientific investigation into their bioactivities and molecular mechanisms of action in order to serve as a starting point for future therapeutic development. In order to determine the pharmacokinetic-dynamic functions of pharmacologically active biomolecules in standardised extracts of A. calamus, more systematic, well-designed, and multi-center clinical trials are required.

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