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PRELIMINARY ANALYSIS OF PHYTOCHEMICALS ANDANTIOXIDANT POTENTIAL OF CONE AND SEED OF *CUPRESSUS SEMPERVIRENS* L.

ARUSA AFTAB[1*], ZUBAIDA YOUSAF[1], TAIBA SALEEM[1], FARAH KHAN[1]

[1]*Department of Botany, Lahore College for Women University Lahore, Pakistan.*

**Corresponding author's email:arusakashif@gmail.com*

Abstract

The study was planned to pharmacologically analyze the cone and seed of *Cupressus sempervirens* L. For this purpose, the plant material (seed/cone) was collected from Malakand Division, dist. Swat. The material was dried and crushed at room temperature. The extraction of powdered plant material was done using non-polar and polar solvents (Petroleum ether, chloroform, methanol and distilled water) through maceration technique. Different phytochemical tests were conducted on the plant extracts to check the pharmacological potential of plant. Some of the tests like alkaloids, saponins, terpenoids and cardiac glycosides gave positive result, while others like anthraquinones and phlobatannins were not detected in the plant. Positive results indicated the presence of secondary metabolites in plants. Secondary metabolites was present in the plant. The chloroform extract of *C. sempervirens* L. seed showed the highest antioxidant value among all the extracts as compared to standard α-Tocopherol and Butyl hydroxyl toluene, whereas distilled water extract of *C. sempervirens* L. seed showed the lowest antioxidant value.

Keywords: Antioxidant potential, Cone, Pharmacological, Phytochemical, Seed, Terpenoids.

Introduction

The folk medicines are based on plant resources globally (Smitherman *et al.*, 2005). The people living in particular areas depend on the use of plants or plant parts indigenously to cope with their basic requirements and frequently have significant information on their usages. It has been assessed that approximately 90% of the world's population in developing countries depends primarily on herbal medicine for basic health care needs (Rizwana and Ghufran, 2007). The plant resources are vigorously increasing having no side effects, easily approachable at cheaper rates and at some extent the only health care source available to the poor (Acharya and Acharya, 2010; Acharya *et al.*, 2009).

Pakistan is rich for having more than 6,000 species of higher plants, 12% of which are used medicinally. Globally medicinal plant and aromatic plants market was at $62 billion in 2002 and may be upto $5 trillion till 2050 (Shinwari, 2010). Medicinal plants contain synergistic and/or side-effect counteracting mixtures of vigorous compounds, hence they are used excessively (Gilani and Atta-ur- Rahman, 2005).

Antioxidants cooperate in oxidative stress reduction in plants. Some of these dietary antioxidants play a protective role in lowering the risk of chronic degenerative diseases, which cause due to oxidative stress (Guerin *et al.*, 2003).Several nuts are the dietary plants have high antioxidant content e.g. tree nuts, walnuts, pecans and chestnuts (Rune *et al.*, 2007).

There are certain phytochemicals in plants that provide attractive colors and fragrance to them. These may be tannins, flavonoids, glycosides, saponins, steroids and alkaloids. *Emblica officinalis*, *Acacia catechu*, *Acacia concina* and *Hibiscus rosa-sinensis*, are medicinal dye yielding plants belong to different families. The qualitative analysis carried out for these plants indicated that saponins, tannins, anthraquinones, flavonoids, terpenoids and alkaloids were found in all the plant except phlobatannins that were only detected in *Acacia catechu.* The petroleum ether and chloroform extract of *Emblica officinalis* does not show potential for oil and fat components, whereas all the extract of *Emblica officinalis* showed positive test for carbohydrates (Monika *et al*.,2013).

Cupressus sempervirens, the Mediterranean Cypress is specie of cypress native to eastern Mediterranean region, in north-east Libya, south-east Greece, Southern Albania, Southern Turkey, Northern Egypt, Western Syria and Israel. It is a coniferous ever green tall tree to 35m (115 ft) tall, aconic crown at top, spread in an area of about 3 to 6 feet, with level and alternate branches. Its life span is large, approximately 1,000 years old (Suresh *et al.*, 2010).

Figure 1: *Cupressus sempervirens* depicting (a) cone and (b) seed.

The chemical composition of essential oil obtain from *C. sempervirens* are monoterpene hydrocarbons, sesquiterpene hydrocarbons, monoterpenoids, oxygenated monoterpenes, oxygenated sesquiterpenoids in the leaves, α-pinene, β pinene, cedrol, myrcene, δ-3-carene, terpinolene, limonene, biflavonoids, diterpenic acids, tannins and proanthocyanidolic oligomer derivatives (Khadija *et al*., 2010; Asgary *et al*., 2013). They also contain bronyl acetate, α-terpinyl acetate, β-Caryophyllene, α-humulene (Hassanzadeh *et al.,* 2004). The study was planned to check medicinal worth of *C. semperverens* L. through phytochemical analysis and antioxidant evaluation.

Materials and Methods

The present investigation was done to evaluate the pharmacological potential of *C. sempervirens* L. The cone and seed of *C.sempervirens* were collected from Malakand, Dist. Swat. The extraction of *C. sempervirens* L. (seed/cone) was performed by using different solvents (mention that solvents). All the extracts were stored at 4ºC.The cone and seed of *C. sempervirens* were dried under normal conditions. The dried plant material was then ground to fine powder, preserved in the amber colored specimen jars, until used. 10 grams of dried plant sample (i.e. cone and seed) were extracted sequentially in non-polar and polar solvents (petroleum ether, chloroform, methanol and dist. water).The extraction was carried out using maceration method. The residue was filtered and the filtrate was preserved for all solvent extracts.

Phytochemical analysis: Phytochemical analysis of the extracted crude extracts of *C. sempervirens* was done using procedure described by Edeoga *et al.* (2005) and Parekh and Chanda (2007).

Antioxidant Activity: The antioxidant evaluation was done through total antioxidant capacity assay according to the methodology of Prieto *et al.* (1999). 1.9ml of reagent solution (0.6M sulphuric acid, 28Mm sodium phosphate and 4mM ammonium molybdate) was mixed with 0.1ml of extracts (0.5mg/ml). The incubation of reaction mixture was done at 95°C for 60 minutes and turned it down to room temperature. Sample absorbance was measured at 695nm against blank BHT (Butyl hydroxyl toluene).Antioxidant activity was expressed as sample absorbance. The antioxidant activity of BHT (0.5mg/ml) was also assayed for comparison.

The data was further analyse doing two ways completely randomize design of ANOVA (Analysis of Variance), to have an idea about the least significant difference among means and treatments. Duncan's multiple range tests was applied for analysis and thus the results were tabulated (Steel andTorre, 1984).

Results and Discussion

Pharmacological activities: Qualitative phytochemical analysis of the *C. sempervirens* L. showed different results.
Alkaloids: In the phytochemical study of *C. sempervirens* L. cone and seed samples gave the end point as creamish precipitate and confirmed the presence of alkaloids.
Saponins: Indication of persistent froth in the plant extract of *C. sempervirens* L.cone and seed showed that saponins were present in respective plant samples.
Coumarins: In the phytochemical analysis of *C. sempervirens* L. cone and seed samples gave the end point as creamish precipitate and confirmed the presence of alkaloids.
Saponins: Indication of persistent froth in the plant extract of *C. sempervirens* L.cone and seed showed that saponins were present in respective plant samples.
Coumarins: In the phytochemical analysis of *C. sempervirens* L. seed and cone extracts there was no emission of yellow inflorescence when placed under UV light which indicated that coumarins were absent in respective plant sample.
Terpenoids: In the phytochemical study of *C. sempervirens* cone and seed gave the end point as brown ring which confirmed the presence of terpenoids.

Antioxidant assay: The plant extracts were evaluated for total antioxidant capacity. The results of the plant extracts were compared with available standard antioxidants. Some of the extracts showed values very much closer to the standard ones. Hence they can be used as standards. The chloroform extract of *C. sempervirens* L. seed showed the highest antioxidant value i.e. 0.772 ± 0.13^{ab} among all the extracts, while the distilled water extract of *C. sempervirens* L. seed have lowest antioxidant value i.e. 0.496 ± 0.22^{b}.In case of *C. sempervirens* L. cone the highest antioxidant value was found in petroleum ether extract i.e. 0.827 ± 0.92^{a}. While the lowest was found in methanol extract of cone i.e. 0.479 ± 0.24^{a}.The methanol extract of *C. sempervirens* L. seed showed the value i.e. 0.656 ± 0.19^{ab} which is closer to the value of standard chemical α-Tocopherol its value is 0.513. While the distilled water extract of the seed have the value 0.496 ± 0.22^{b} which is close to standard chemical BHT whose value is 0.476 as depicted in figure 2. So both can be used in place of the standard chemical because it showed greatest trend towards the standard.

The chloroform extract of *C. semperirens* L. cone showed the value, 0.571 ± 0.21^{a} which is close to the value of standard chemical α-Tocopherol i.e. 0.513. It means that the chloroform extract of *C. sempervirens* L. can be used as an alternative to the standard chemical because it is very much comparable with the standard. While the methanol extract showed the value, 0.479 ± 0.24^{a} this is close to the value of standard chemical BHT, 0.476. So it can be used in place of the standard chemical because it shows great trend towards the standard (figure 3).

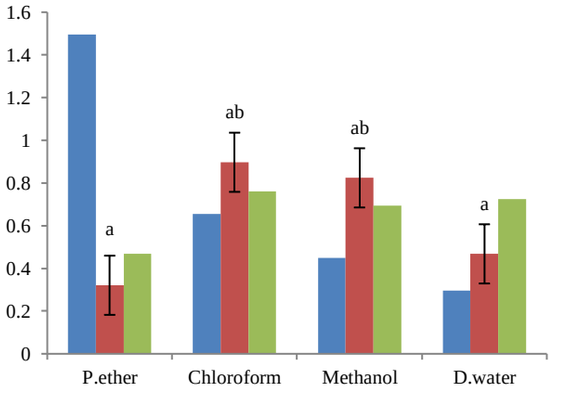


Figure 2: Antioxidant values of various extracts of *C. sempervirens*L. seed in different solvents**.**

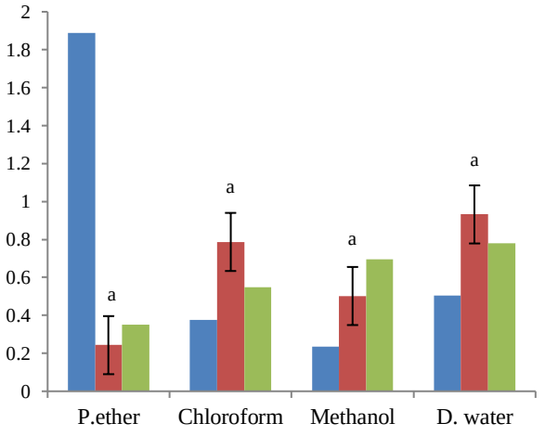
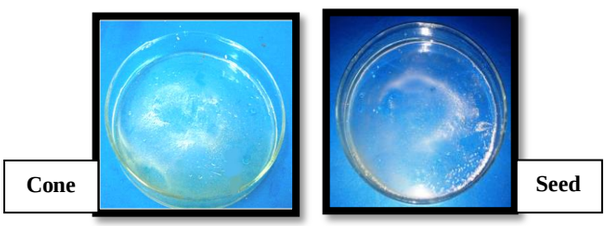
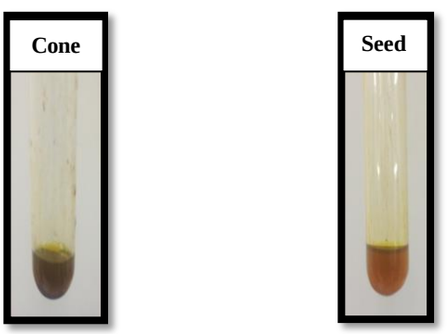


Figure 3: Antioxidant values of various extracts of *C. sempervirens* L. cone in different solvents.



Alkaloids Detection

Saponins detection test



Quinones detection test Terpenoids detection test

Medicinal plants are the primary source of medicines for the treatment of human diseases in many rural areas of the developing countries (Chitme*et al*., 2004). About 80% of the world population relies on the traditional medicine for their primary health care (Owolabi and Omogbai, 2007). The medicinal value of the plant is due to the presence of various bioactive chemical constituents such as alkaloids, tannins, flavanoids and phenolic compounds. Therefore, the plants with the medicinal values have to be investigated to understand their safety and efficacy (Nascimento *et al*., 2000).A qualitative phytochemical analysis was performed for the detection of alkaloids in *Ocimum sanctum* (Tulsi) and *Azadirachtaindica* (Neem) (Biswas *et al*., 2002). The phytochemical screening of the stem bark extract of *A. indica*and and *O. sanctum* revealed presence of alkaloids. Same is the case with *C. sempervirens* L. cone and seed, the creamish color indicated the presence of alkaloids when treated with Mayor's reagent.

In the present activity both plant powders (cone and seed) was treated with 80% ethanol separately and mixed with 1% KOH, the production of dark yellow color indicated the presence of flavonoids. Likewise, the work was done by Maluventhan *et al.* (2010); their experiment also showed the presence of flavonoids in the ethanol, chloroform and aqueous extracts of *Cardiospermum halicacabum* (ballon vine) leaves. The phytochemical study was done to determine the presence of saponins in *C. sempervirens* L. which is demonstrated by the production of persistent froth in the boiled plant extract of distilled water. The same experiment was conducted by Atangwho *et al*. (2009) on *Vernonia amygdalina*, using standard methods and was compared. Among all the phytochemicals saponins were significantly predominant in *V. amygdalina*.

Study was carried out on the presence of medicinal active constituents *in Aloe vera* and their phytochemically active compounds were qualitatively analyzed. In this screening processterpenoids were present in the plant under study (Arunkumar and Muthuselvam, 2009). Same is the case with our experiment conducted on *C. sempervirens* L. The plant extract was dissolved in 2 ml of chloroform and then acetic acid and conc. H_2SO_4 was added, the end point as blue green ring displayed the presence of Terpenoids. Here in our present study the exploration of the phytochemical constituent was done to detect the presence of anthraqquinones. For this the plant extract was treated with 1% of HCL and 2ml of benzene and 10% NH_4OH, the emergence of pink violet or red colour confirmed that anthraquinones were present.

The same qualitative study was done by Kumar *et al*. (2009) on the different plant extract of seed of *Syzgium cumini* (Jaman) they showed the presence of anthraquinones.

The phytochemical examination was done on under study plant *C. sempervirens* revealed the presence/absence ofcoumarins. The moistened plant extract was covererd with filter dipped in 0.1N NaOH and place it in boiling water for few minutes, after it when the filter paper was examined under Ultra-violet (UV) light no yellow flouresence was observed on filter paper indicating that coumarins were absent in both parts of our respective plant sample. The qualitative phytochemical study was organized by Savithramma *et al.* (2011) on some medicinal plants like *Clematis gouriana*Roxb, *Cleome viscose* L. *Cochlospermum religiosum* L.Theplant showed the presence of important phytochemicals like coumrains.

When further phytochemical analysis was done on *C. sempervirens* L., the plant powder was boiled with 1% aqueous HCL the formation of red precipitate proved that phlobatanins

were present. The same methodology was conducted by Arunkumar and Muthuselvam (2009) on *Aloevera* and their result showed that phlobatanins were absent. For the identification of tannins the same experiment was conducted for the same plant *Ocimum sanctum* (Tulsi) and *Azadirachta indica* (Neem). The phytochemical identification showed the presence of tannins (Biswas *et al.*, 2002). While in *C. sempervirens* L. cone and seed when the plant powder was boiled with distilled water and added 1% $FeCl_3$, solution converted into brownish color which showed the presence of tannins.

Phytochemical experiment carried out on plant extract of *C. sempervirens* L. revealed that cardiac glycosides were present in the plant, when blue-green color developed on addition of 2 ml of glacial acetic acid and $FeCl_3$ and H_2SO_4 (Figure 4) In the same way Javed *et al.* (2012) took in consideration the phytochemical constituent of essential oil, aqueous, methanol and chloroform extract of *Eucalyptus citridora* hook leaves, a qualitative phytochemical analysis showed that methanolic extract have the entire identified biochemical constituent. With the exception, chloroform extracts have a significant amount of cardiac glycosides. The results showed that all cone and seed are pharmacological to some extent. Some of them showed very high pharmacological activity, while some of them are comparatively less in nature.

Conclusion

Pharmacological analysis was done on *C. sempervirens* L. cone and seed. For this purpose different test were conducted on the plant. Some of the test gave positive result while some of them gave negative results. Positive result showed that secondary metabolites are present in the plant. These secondary metabolites provide strong defense system to the plant. So it can be concluded that in future plant can be used in pharmaceutical industry for various pharmacological purposes.

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