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Antioxidant and Phytochemical composition of Leaves, Stem and Root Extracts of *Withaniacoagulans* and *Withania somnifera*

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ABSTRACT
For biochemical research, various Withania coagulans and Withania somnifera plant sections from the Solanaceae family were used. Although they are mainly regarded as dangerous plants and only occasionally utilized in Pakistani folk medicine, these are wild plants. The yield of menthol extracted from the roots, stems, and leaves of both plants was also calculated. W. coagulans leaves had a higher extract yield percentage (7.6%) than the stem (6.3%) or root (6.3%). All portions of W. coagulans had a higher percent extract yield than the same parts of W. somnifera. For W. somnifera and W. coagulans, the extract yield pattern is comparable in terms of percentage. It is higher in the leaves (5.5%) and falls from the stem (5.2%) to the roots (4.7%). Through spectrophotometric analysis, flavonoids and total phenolics were determined using the Folin-Ciocalteu reagent and aluminum chloride reagent. W. coagulans leaves contained the highest amount of total phenolics and flavonoids (58.21 mg GEA/g and 47 mg RE/g). Total phenolic and flavonoid content decreases in a comparable manner from extracts of the leaves to those of the stem and roots. Extracts from both plants' various sections were measured for their antioxidant activity at various levels. Antioxidant activity is higher in all portions of W. coagulans than it is in W. somnifera. These findings show that both species include valuable biome- dical elements that could be incorporated into the creation of contemporary drugs.

Keywords-: *Medicinal plants, secondary metabolites, therapeutic agents, ashwagandha,*

I. Introduction

All across the world, different illnesses are treated with plant-based medications. The majority of these plants normally flourish in the wild. Wild medicinal plants have a vital role in both domestic and international trade (Sureshkumar et al., 2017). The indigenous population learned how to use plants as medicine through trial and error, and this knowledge has been passed down from generation to generation. Because they have fewer side effects, using plants as medications is seen as being more environmentally and human-friendly (Sisubalan et al., 2014; Azhar et al., 2014). Plants in Pakistan and other developing nations where agriculture is the primary source of income are more than just an ecosystem's balancing act. In addition, they provide food, fuel, medication, and animal feed (Azhar et al., 2015). Plants themselves are potent medicinal agents due to the presence of beneficial phytochemicals and antioxidants. The oxidative components of medicinal herbs can reduce tissue damage (Pourmorad et al., 2006). Even plants and a wide variety of other plants have the potential to be good antioxidants. The Solanaceae family includes 90 genera of annual, biennial, and perennial plants, as well as 3000–4000 different species of herbs, shrubs, and trees. 14 genera and 400–600 species are thought to occur in Pakistan. This family of plants includes many extensively used ethno-herbal plants with a variety of secondary metabolites (Shaheen et al., 2015). Alkaloids, flavonoids, and terpenes found in Solanaceae plants have significant implications for the global herbal industry.

Both Withania coagulans and Withania somnifera are wild plants that belong to the Solanaceae family (Shah et al., 2013). They have successfully adapted to the dry regions of Pakistan. Ash-wagandha, or W. somnifera, is well-known. According to Gupta (2012), W. coagulans is also known by the common names Akri (Hindi), Khamijria (Punjabi), and paneerband in Hindi. The W. somnifera plant can grow as tall as 1.50 m (Umadevi et al., 2012). With yellowish-red seeds, ovate-shaped green leaves, and light brown roots. W. somnifera supports mental health and resilience to natural impacts. It treats a variety of ailments, including anxiety, mental illness, stomach discomfort, inflammation, bacterial infections, and pregnancy in both humans and animals. W. somnifera's roots, stem, and roots are all significant medical components. W. coagulans has the

ability to cause milk to coagulate in fruits, which is due to the withanin enzyme found in the pulp and husk. has use as an antibilious, anti-asthmatic, anti-inflammatory, emetic, diuretic, sedative, CNS depressant, and in the treatment of chronic liver problems.

Proteins, carbohydrates, vitamins, minerals, enzymes, alkaloids, terpenoids, flavonoids, quinines, phenols, and carotenoids are just a few of the important phytochemicals that may be found in plants and are excellent therapeutic agents (Rajamano- haran, 2013; Al-Rifai et al., 2017). Due to their therapeutic benefits, herbal medicines are used to create new medications (Shah et al., 2013; Yuan et al., 2016). Herbal plants have chemicals that can be used to create extremely effective and powerful medications (Srivastava et al., 1996). The existence of biological or pharmacological components in these herbal plants is then scientifically screened after being identified from local communities using their traditional medicinal methods. These phytochemical studies give herbal and dietary supplement manufacturers medicinal information (Uniyal et al., 2006; Javid et al., 2017). Different plant sections from the two mentioned native species were analyzed chemically to identify secondary metabolites and antioxidant components in the Cholistan Desert, Punjab.

II. Material and Method

Plant sample collection

Different parts (stem, leaves and root) ofdisease free *W. coagulans* and *W. som-nifera* plants from various areas of Cho-listan desert were collected. All the plantparts were completely washed in running water for thrice and finally distilled water was used and dried under shade. All the samples were grinded with an electric grinder. Powdered samples were storedin air tight vase. The apparatus used was also cleaned and washed with distilled water for removal of unprocessed mate-rial and other contaminations.

Extract ratio of plant parts

300 gm plant's refined powder wasmixed with methanol in a container and placed soaking for 14 days. Solution was filtered and concentrated on rotaryevaporator. The collected solvent samples were measured to collect crude extract at temperature range 40–45°C.

Total Phenolics and flavonoidscontent

Total Phenolic contents (TPC) and fla-vonoids contents were measured by Spectrometry method. The spectro- photometric showed values in nm fromwhere phenolic contents were calcu-lated (Khoddami *et al.,* 2013). The ext- racts of plant parts were mixed in Gallic acid, sodium carbonate and Folin-Cio- calteu reagent. The spectrophotometerreadings at 765nm after 15 min and at725nm after 30 min were recorded. Va- lues were measured in Gallic acid units(Raj *et al.,* 2017). Colorimetric methodwas used for flavonoids where a chemi-cal aluminum chloride was also added. Different parts extracts were amalgama-ted with potassium acetate and alumi-num chloride. Visible spectrophotome-ter readings were 415 nm after 30 min and 510 nm after 15 min. Absorbance of this mixture was determined. Flavono-id contents were assessed in mg RE/gwhile Absorbance of Gallic acid contentwas noted and TPC measured in mg GEA/g (Senguttuvan *et al.,* 2014).

Determination of Antioxidantactivity

Antioxidant activity was measurement by DPPH (2, 2-diphenyl-1-picryl-hydrazyl- hydrate) method by using ascorbic acidas standard (Chikhi, *et al.,* 2012; Ilahi *etal.,* 2013). A stock solution was preparedby adding analytical grade methanol inweighted amount of the methanolic cru-de extracts of all parts of both plants. Both plant samples of root, stem, leavesin different concentrations (100, 200,

400, 600, 800ppm) were prepared in methanol from stock solution. Similarly, ascorbic acid samples with same con-centrations were prepared. Methanol as solvent was used to prepare DPPH(0.002%) solution. 2 ml of DPPH soluti-on was added and dissolved separatelyin standard solution (ascorbic acid) and2ml concentrated sample. This resulted solution was incubated for half an hour for measuring optical density at 517 nm. The control contains methanol only. The % scavenging activity (inhibition percentage) was measured by the formu-la given (Senguttuvan *et al.,* 2014). Here, $A_{Control}$ and $A_{Extract}$ represent the optical density of extract sample andcontrol reaction respectively.

III. Result and Discussion

Phytochemical Analysis

All medicinal plants have high quanti-ties of different phytochemicals. Thesephytochemicals were tested in this re-search work. The two plants *W. coagu-lans* and *W. somnifera* of family solana-ceae were investigated for determining extract ratio in leaves, stem and roots,antioxidants and flavonoid and total phenolic content.

$$I\,\% = [AControl - AExtract) / AControl] \times 100$$

Extract yield

The % of extract yield of leaves of *W.coagulans* was recorded high (7.6 %) than stem (6.3 %) and root (6.3 %) as shown by Fig. The % extract yield of all parts of *W. coagulans* was greater than same parts of *W. somnifera*. The pattern of% extract yield is similar indifferent parts of *W. somnifera* as in *W. coagulans*. It was higher in leaves (5.5 %) and a decreasing trend from stem (5.2 %) to roots (4.7 %). Variation in extraction yield of differentplant parts is dependent on various factors as functions, presence of freshmaterial, food storage and fertility of % age yield of different extracted plant portion of *W. Coagulans* and *W. Somnifera* different plant parts. Variation in diffe-rent plants may be due to genetic ma-keup and soil chemistry.

Flavonoids contents

The flavonoids contents were determi- ned by colorimetric method. Flavonoids contents for leaves, stem and root werecalculated. Flavonoids contents in *W. coagulans* leaves were noted as 47.0 mgRE/g and for W. *somnifera* leave 43.51 mgRE/g. The high flavonoid contents werepresent in the leaves, stem and rootsof *W. coagulans* in comparison with the same plant parts of *W. somnifera* (Tab. 1).There is a decreasing trend of flavono- id contents of *W. coagulans* ftom lea-ves to roots. Similarly, in *W. somnifera*flavonoids content were higher in lea- ves and lowest in roots.

Presence of flavonoids exhibits the antioxidant activity of that plant and its concentration is greatly affected by biological, genetic diversity, environ- mental and temporal variations in dif- ferent plants (Kumar, *et al.*, 2018).

Total Phenolic Contents

TPC of all parts of both species weredetermined and expressed as mg gal-lic acid per gram (dry weight) in table 1. The higher value of TPC was measu-red in leaves of *W. coagulans* (58.21 mg GEA/g) than its stem and roots i.e., 26.25 mg GEA/g, 15.95 mg GEA/g. In *W. somnifera* TPC were higher in leaves(53.53mg GEA/g) similar as in *W. coagu- lans* leaves but lower than the later. These Phenolics in plants are im- portant constituents with propertieswhich exhibit antioxidant activities(Kumar, *et al.*, 2018).

Plant Species	Part	TPC* (mg GEA/g)	Flavonoids (mg RE/g)
W. coagulans	Leaves	58.21±0.351	47.00±0.660
	Stem	26.25±0.871	44.41±0.360
	Root	15.95±0.572	42.82±1.189
W. somnifera	Leaves	53.53±0.537	43.51±0.346
	Stem	15.95±0.572	42.82±1.189
	Root	11.60±0.350	39.13±0.607

* *Total Phenolic Contents*

Antioxidant activity in leaves Antioxidant activity results obtained are statistically presented as ANOVAgraph in Figure 2. Graph comparisonreveals different concentrations ofleaves extracts of both species. The higher activity value was observed in *W. coagulans* (43 %) than *W. somnifera* (39.5 %) by same 800 ppm concentra- tion. Same value (35.5 %) was shown in leaves of *W. coagulans* and *W. som*- Anitioxidant activity comparision for varoius concentrations of leaves extract.

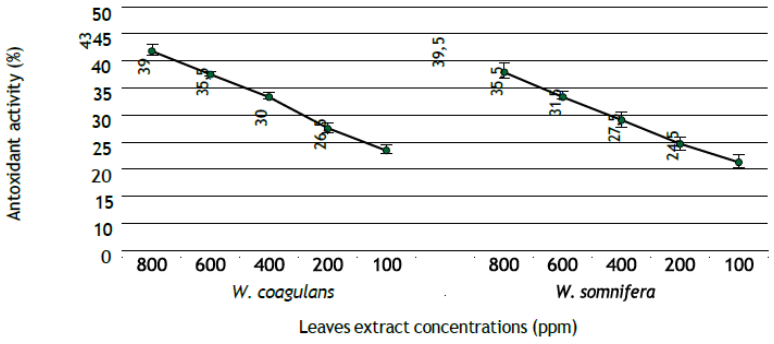

Fig. 2: Antioxidant activity for various concentration of leaves extract of both plants.

nifera by different concentrations i.e.,400ppm and 600 ppm. Both species showed lowest activity value of 26.5 % (*W. coagulans*) and 24.5 % (*W. somni-fera*) respectively in 100 ppm.

Antioxidant activity in stem

The antioxidant activity stem at diffe-rent concentrations for both species was determined. *W. coagulans* show-ed a maximum activity (65 %) than *W. somnifera* (62.1 %) at 800 ppm. The lowest antioxidant activity of both species was noted as 43.9 % for *W. co-agulans* and 41.23 % for *W. somnifera* at 100ppm lower concentration. Stem extract of *W. coagulans* exhibits higher activity than *W. somnifera* at all con- centrations (Fig.)Antioxidant activity in root Antioxidant activity results were stati- stically presented as ANOVA graph in fig. 4. A graphical representation of antioxidant activity in root extract of bothspecies indicated a higher activity in *W.coagulans* (69.3 %) than W. somnife- ra (66.1 %) by 800ppm concentration. In lower concentration i.e., 100 ppmboth species showed lowest activity of 47.5 % for *W. coagulans* and 45.3 % for *W. somnifera*. Roots of *W. coagulans* exhibits higher activity at all concentra- tions than the other counterpart. Phenolics and flavonoid compounds are significant antioxidants whichhave ability to deactivate free radicalsas tendency of donating hydrogenatoms in free radical process. Theirideal structural formation is helpfulin free radical scavenging (Amarowicz*et al.*, 2004). Different studies indica-ted a linear correlation between totalphenolic, flavonoid content and an-tioxidant capacity (Aryal *et al.*, 2019).This study reveals the presence of se-condary metabolite like phenolics andflavonoids in all parts of *W. coagulans* and *W. somnifera* and antioxidants. These plants may be an important source of vital natural antioxidants. These plants showed a significant an-tioxidant activity, thus must broughtunder consideration for pharmaceuti-cals. The studied parameters were theassessment of Phenolics, flavonoidscontents and antioxidant properties,and not disease-specific but this studymay guide further investigations.

IV. CONCLUSION

All portions of W. coagulans had a higher percent extract yield than the same parts of W. somnifera. For W. somnifera and W. coagulans, the extract yield pattern is comparable in terms of percentage. It is higher in the leaves (5.5%) and falls from the stem (5.2%) to the roots (4.7%). Through spectrophotometric analysis, flavonoids and total phenolics were determined using the Folin-Ciocalteu reagent and aluminum chloride reagent. W. coagulans leaves contained the highest amount of total phenolics and flavonoids (58.21 mg GEA/g and 47 mg RE/g). Total phenolic and flavonoid content decreases in a comparable manner from extracts of the leaves to those of the stem and roots. Extracts from both plants' various sections were measured for their antioxidant activity at various levels. Antioxidant activity is higher in all portions of W. coagulans than it is in W. somnifera. These findings show that both species include valuable biome- dical elements that could be incorporated into the creation of contemporary drugs.

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