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COMPARATIVE ANTIOXIDANT ACTIVITY OF *HYPTIS SUAVEOLENS* BEFORE AND AFTER HYDRODISTILLATION

***Anita Sharma[1], Anu Sharma[2], Daizy R. Batish[3]**

[1,2,3]*Department of Botany, Panjab University, Chandigarh 160014, (India)*

ABSTRACT

In the present study the comparative antioxidant activity of aqueous extracts of Hyptis suaveolens plant material before and after hydrodistillation was evaluated with a view to finding replacement for the synthetic antioxidants. For this, aqueous extracts were prepared from the plant powder (PP) before and after hydrodistillation (left over residue powder or RP). The antioxidant activity of both the aqueous extracts (PP and RP) were evaluated by various antioxidant assays like 2, 2-diphenyl-1-picrylhydrazyl (DPPH), hydroxyl radical (OH) scavenging hydrogen peroxide (H_2O_2) and FRAP activity. Along with these assays, total phenolic content (TPC) and total flavonoid content (TFC) were also calculated from the PP and RP. Further, the correlation between TPC and TFC of PP and RP were correlated with their antioxidant activities and it shows a strong positive correlation between them. At last, it was concluded that the by-product of the distillation could be valorizing as a source of antioxidant for food and pharmaceutical industries.

Key words: Antioxidant activity, Hyptis suaveolens, Total phenolic content, Total flavonoid content.

I.INTRODUCTION

Free radicals may be defined as any chemical species that are capable of existing with one or more unpaired outer shell electrons. They are extremely reactive and generally highly unstable [1]. These are extremely reactive and potentially damaging chemical species that are produced either from the external sources like ionizing radiation, tobacco smoke, pesticides, pollutants or internally in the cells of living beings as byproducts of various metabolic activities[1]. At low/moderate concentration, these acts as a secondary messenger in intracellular signaling cascades that mediate several responses in cells, however, at high concentration, cause damage to biomolecules (i.e. lipids, proteins and nucleic acids) and leading to subsequent cellular death by modes of necrosis or apoptosis. There are several reports that show that free radicals produced molecular alterations that are associated with various degenerative human diseases such as arteriosclerosis, cancers, Alzheimer's disease, Parkinson's disease, diabetes, asthma, arthritis, immune deficiency diseases and aging [2,3,4,5]. Therefore, to combat these there are many beneficial compounds are produced to protect the body against these free radicals known as antioxidants. Antioxidants are substances that mop up free radicals and prevent them from causing cell damage [6]. During the present times, endogenously produced antioxidants are not sufficient since humans are continuously exposed to pollutants / other toxins. To overcome their negative effects, consumption of a diet rich in antioxidant compounds or exogenous supply of synthetic antioxidants is required. However, there is widespread agreement that some synthetic antioxidants such as butylhydroxyanisole

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(BHA) and butylhydroxytoluene (BHT) were found to be toxic and carcinogenic in animal models [7,8,9]. Therefore, these synthetic antioxidants need to be replaced with safer and inexpensive antioxidants of natural origin.

In the exploration of natural sources offering biologically active compounds, recent studies have shown that medicinal aromatic herbs are the most promising and diverse source of natural antioxidants. Plants naturally produce a wide array of secondary metabolites (antioxidants) which not only protect them from environmental and oxidative stresses but also contribute towards their flavor and sensory properties. These secondary metabolites have the capacity to retard the progress of various chronic diseases act as natural antioxidants [10]. The aromatic herbs particularly belonging to family Lamiaceae have been found to be very effective with regard to natural antioxidants. In various studies, several aromatic plants like rosemary, sage, oregano, and thyme have shown strong antioxidant activity [11, 12]. The aqueous herbal extracts have attracted attention since they can be consumed on a daily basis as decoctions. The extracts of various plants of this family have been extensively used in traditional diet and popular as medicine [13,14,15]. Their therapeutic actions are assigned to biologically active polyphenol components, such as flavonoids and phenolic acids, which possess antioxidant activities [16,17,18].

Hyptis suaveolens (L.) Poit. commonly known as Bush mint or Pignut, is an aromatic herb also belonging to family Lamiaceae. It is native to tropical America but now it occurs in various parts of the world including India. In India, it grows as a weed along the rail tracks, roadsides or foothills of open forests, forest clearings [19, 20]. The plant is medicinally important and used as a stimulant, carminative, for curing wounds, against infection of the uterus, parasitic skin diseases etc. Different parts of the plant have been used by traditional healers in the treatment of various ailments and disease conditions. The leaves decoction of the plant is used for treating boils, eczema, and diabetes mellitus [21, 22]. The paste of freshly crushed leaves is applied on the forehead to treat headaches. However, an infusion made from the leaves and its inflorescence was used as a stimulant, carminative, diuretic and antipyretic [23]. A decoction of the whole plant is also used to alleviate diarrhea and various kidney ailments. The plant has been also reported to rich in phytonutrients such as alkaloids, tannins, saponins, flavonoids and, terpenoids [24, 25]. Besides these properties its aerial parts were rich in essential oil and several studies have also show that the essential oils of *H. suaveolens* possess various biological activities such as antifungal [26], antibacterial [27] anticonvulsant [28] and antioxidant activity [29]. Although, it's essential shows various activities, however, its extraction residues have not yet been investigated. As till now, no study was conducted on that left over residue that's going as waste during the process of essential oil extraction. A study was therefore conducted with a view that residue might also possess some phytochemicals and the activity of the residue was compared with the plant material before hydro-distillation. Therefore, it was the first time a comparative antioxidant activity of *H. suaveolens* the plant material was observed before and after hydro distillation.

II. MATERIAL AND METHODS

2.1 Plant material: The aerial parts of wildly grown plants of *H. suavolens* were collected from the outskirts of Chandigarh (India). A voucher specimen of the plant was deposited (PAN# 18048) in the herbarium of Botany department, Panjab University Chandigarh.

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2.2. Preparation of Extracts: The aqueous extracts were prepared from the dried powder of aerial parts before hydro distillation of *H. suaveolens* (known as plant powder or PP) of and after hydro-distillation the left over plant residue (known as residue powder or RP). For this, one gram powder either PP or RP was soaked in 100 ml of water for overnight. After soaking, it was filtered, and the filtrate was termed as water extract. From this 1%, filtrate solutions of PP and RP, further concentrations i.e. 0.5, 0.25 and 0.1% were prepared by dilution with distilled water.

2.3. Estimation of total phenolic and flavonoid content: The total phenolic content was determined as per the method is given by [30] with gallic acid as equivalent used as a standard. The amount of total flavonoid content was determined by dowd method as per given by [31] and quercetin was used as a standard.

2.4. DPPH (2, 2-diphenyl-1-picryl hydrazyl) radical scavenging Activity: The DPPH scavenging activity was measured as per the method is given by [32] with slight modification. Various concentrations of sample solutions of water extract PP and RP (0.1 to 1%) were mixed with 1ml of 0.1Mm alcoholic DPPH solution. After this, samples were incubated in dark at room temperature. The absorbance was measured at 517 nm. Decreased in absorbance of the samples with increasing concentration indicates DPPH scavenging capability of PP or RP. The percent scavenging was calculated by using the formulae shown below:

DPPH scavenging (%) = $[(A_{control} - A_{sample}) / A_{control}] \times 100$

2.5. Hydroxyl radical scavenging activity: For assaying the hydroxyl radical scavenging activity method given by [33] was used. To 0.5 ml of sample solutions water extracts PP and RP (0.1 to 1%), 2.5 ml of the reaction mixture (3 mM 2-deoxyribose, 0.1 mM $FeCl_3$, 1 mM H_2O_2, 0.1 mM EDTA, 0.1 mM ascorbic acid, and 0.02 M phosphate buffer (pH 7.4)) was added to make a final volume of 3 ml and incubated for 1 h at 37°C. Then, 1 ml of tert-butyl alcohol (TBA) and 1 ml of trichloroacetic acid (TCA) were added to the test tubes and these were heated at 100 °C for 20 min. After cooling the mixture, absorbance was read at 532 nm against a blank containing buffer and 2-deoxyribose. The percent scavenging was calculated by using formulae given below:

OH scavenging (%) = $[(A_{control} - A_{sample}) / A_{control}] \times 100$

2.6. Assay for Hydrogen peroxide scavenging (H_2O_2): H_2O_2 scavenging activity was determined as per the method of [34]. A 40 mM solution of H_2O_2 was prepared in a phosphate buffer (pH 7.4). Different concentrations of sample solutions water extracts PP and RP (0.1 to 1%) were added to 0.6 ml of H_2O_2 and the absorbance of the solution was read at 230 nm after 10 min against a blank containing phosphate buffer without H_2O_2. Butylated hydroxytoluene (BHT) was used as a positive standard. The percent scavenging of H_2O_2 was calculated using the formula: H_2O_2 scavenging (%) = $[(A_{control} - A_{sample}) / A_{control}] \times 100$

2.7. Assay for Total antioxidant activity (TAA): The total antioxidant activity of essential oil was evaluated as per the method given by [35]. To 0.1 ml of sample solutions water extracts PP and RP (0.1 to 1%), 1 ml of reagent solution (6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) was added. Then, the reaction mixture was incubated at 95°C for 90 min. The absorbance of the solution was measured at 695 nm against blank using Shimadzu UV-190 double beam spectrophotometer. The antioxidant capacity of the extract was expressed as mg ascorbic acid equivalent per gram of the plant material (mg AE/g tissue).

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2.8. Ferric ion reducing antioxidant power (FRAP) assay: Ferric ion reducing antioxidant power (FRAP) assay was done by the method is given by [36]. Sample solutions water extracts PP and RP (0.1 to 1%) equivalent to 0.2 ml was taken and to this, 0.6 ml of phosphate buffer and 0.6 ml of potassium ferricyanide solutions were added. The mixture was incubated for 30 min at 50ºC temperature. After this, 0.6 ml of Trichloroacetic acid (TCA) solution was added, in each test tube followed by centrifugation for 10 min at 3000 rpm. From this, 0.6 ml of supernatant was taken and to this was added 0.6 ml of distilled water and 0.125 ml of freshly prepared $FeCl_3$ solution. The concentration of FRAP was determined spectrophotometrically at 700 nm and was expressed in optical density directly. Percent inhibition was calculated as given:

FRAP Activity (%) = $[(A_{control} - A_{sample}) / A_{control}] \times 100$

2.9. Statistical analysis: The statistical analysis of the data was performed using SPSS version 16.0 (SPSS Inc., Chicago, IL). A general analysis of variance was performed to check the differences between PP and RP. Further, the difference between the antioxidant activity of PP and RP were significantly represented by applying 2 samples *t* – test at $P \leq 0.05$. To study the relationship between antioxidant activity of PP and RP samples with TPC and TFC was described as Pearson correlation coefficient (*r*).

III. RESULTS AND DISCUSSION

3.1. Total phenolic and Flavonoid content

It has been reported that the aromatic plants act as a source of natural antioxidants due to the presence of phenolic and flavonoids [37, 38]. In the present study, from the results, it was clear that the amount of total phenolic (TPC) and total flavonoid content (TFC) was not much affected by the hydro-distillation method. As it was clearly depicted from the **Fig. 1**, that the amount of phenolic and flavonoid content before (PP) and after hydro-distillation (RP), were ranged from ~ 10 to 9 mg GAE g^{-1} and 2.5 to 1.3 mg QE g^{-1} respectively **(Fig. 1A).** This indicates that the good amount of phenolic and flavonoids are still remaining in the left over residue even after oil extraction. The antioxidant capacity of phenolic compounds is determined by their structure, in particular, the ease with which a hydrogen atom from an aromatic hydroxyl group can be donated to the free radical. *H. suaveolens* considered as being an important medicinal plant due to the presence of various phytochemicals [39]**.** It possesses medicinal value due to the presence of various kinds of primary (i.e. starch, proteins, tannins, saponins, fats, alkaloids) and secondary metabolites i.e. phenolic and flavonoid [40].

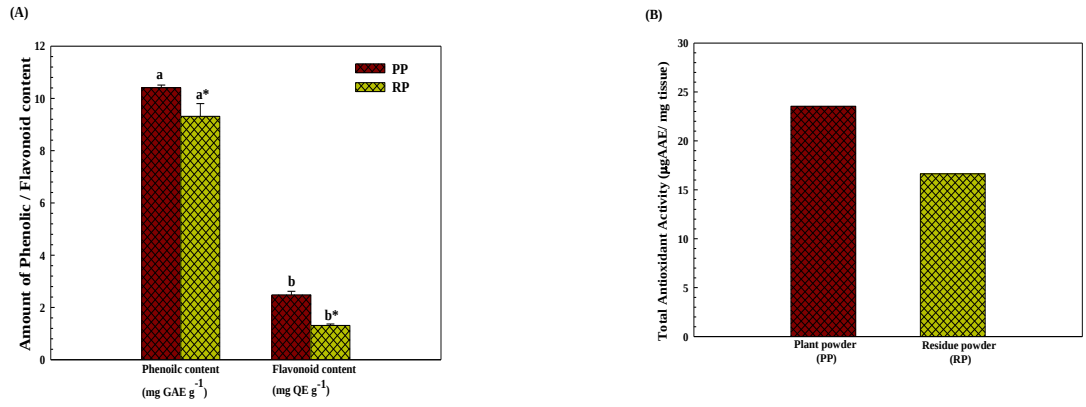

Figure 1: The effect of hydrodistillation on: (A) Total phenolic and flavonoid content and, (B) Total antioxidant activity of *H. suaveolens* aqueous extracts prepared from plant powder (PP) and residue powder (RP). *represents

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significance difference between RP and PP applying 2 samples t– test at $P \leq 0.05$. Different alphabetical letters represented represents significance at $P \leq 0.05$. Bars represent standard error.

3.2. Total Antioxidant Activity (TAA)

The total antioxidant activity is one of the important spectrophotometric methods which is based on the reduction of Mo (VI) to Mo (V) in the presence of antioxidant compounds leading to the formation of the green coloured phosphate molybdenum (V) complex at high temperature and acidic conditions. In the present study, the total antioxidant activity was expressed as µg AAE/ml. Total antioxidant activity of *H. suaveolens* aqueous extracts (PP and RP) are represented in **Fig.1B**. From the results, it was clear that plant powder possesses ~23.54 µg AAE mg^{-1} that is nearly comparable to that of residue powder ~ 17.00 µg AAE mg^{-1}. Further, the correlation coefficient between total antioxidant activity (TAA) and total phenolic content (TPC) and total flavonoid content (TFC) of water extracts (PP and RP) were determined. Both PP and RP shows a significant and strong positive correlation between total antioxidant activity with phenolic (r=0.994 and r=0.993) and flavonoid (r=0.987 and r=0.987) content **(Table.1).** Some previous studies [41] showed that antioxidant activity of plants was correlated with their phenolic and flavonoid content. In the present study also from linear correlation (*r*) values, it was clear that around 98.7-99% total antioxidant activity of PP and RP was due to the presence of phenolic and flavonoid content. These results suggest that 99% of the antioxidant capacity of *H. suaveolens* is due to the contribution of phenolic and flavonoid compounds.

3.3. Hydroxyl ($^{\bullet}OH$) scavenging activity

Hydroxyl radicals are known to be highly reactive and considered as one of the toxic free radicals produced in the living systems. They greatly damage biomolecules and also have a long lifetime [42, 43]. This radical can be formed by Fenton reaction in the presence of reduced transition metals (such as Fe^{2+}) and H_2O_2, which is known to be the most reactive of all the reduced forms of dioxygen and is thought to initiate cell damage *in vivo* [44]. It is thus, very important to find an effective antioxidant or scavenger to combat ROS. In the present study, the antioxidant activity of *H. suaveolens* aqueous extracts (PP and RP) was studied and it was found that the radical scavenging activity of PP and RP were also increased with an increase in concentration. The maximum activity was shown by PP as compared to RP **(Fig. 2D).** Further, a correlation between TPC and TFC of water extracts (PP and RP) with the hydroxyl radical scavenging activity of PP and RP were determined. It showed a strong correlation between TPC and RP and TPC and PP i.e. r=0.99 and r=0.97, respectively. In case of flavonoid content also, it showed a significant correlation between TFC with PP (r=0.98) and TFC and RP (r=0.94). A strong correlation between TPC, TFC with hydroxyl scavenging activity, shows that around 98-99% antioxidant activity of *H. suaveolens* was due to the presence of phenolic and flavonoid content present it.

3.4. Hydrogen peroxide scavenging activity (H_2O_2)

Hydrogen peroxide (H_2O_2) is a non-radical ROS in living organisms and has the ability to penetrate cell membranes, inactivate enzymes by oxidation of thiol groups, and initiate lipid peroxidation. H_2O_2, itself is not very reactive, but it can sometimes be toxic to cells due to rise in hydroxyl radical in the interior environment of the cellular components [45]. Being a strong oxidant it oxidizes other compounds in cells and also induces the generation of hydroxyl radicals, which themselves are not toxic enhance the production of hydroxyl radicals.

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Therefore, removing or scavenging of hydrogen peroxide ((H_2O_2) is very important for antioxidant defense in cell or food systems. The percent scavenging activity of water extracts (PP and RP) were also increased in a dose dependent manner and activity was high in case of PP as compared to RP **(Fig. 2C).**

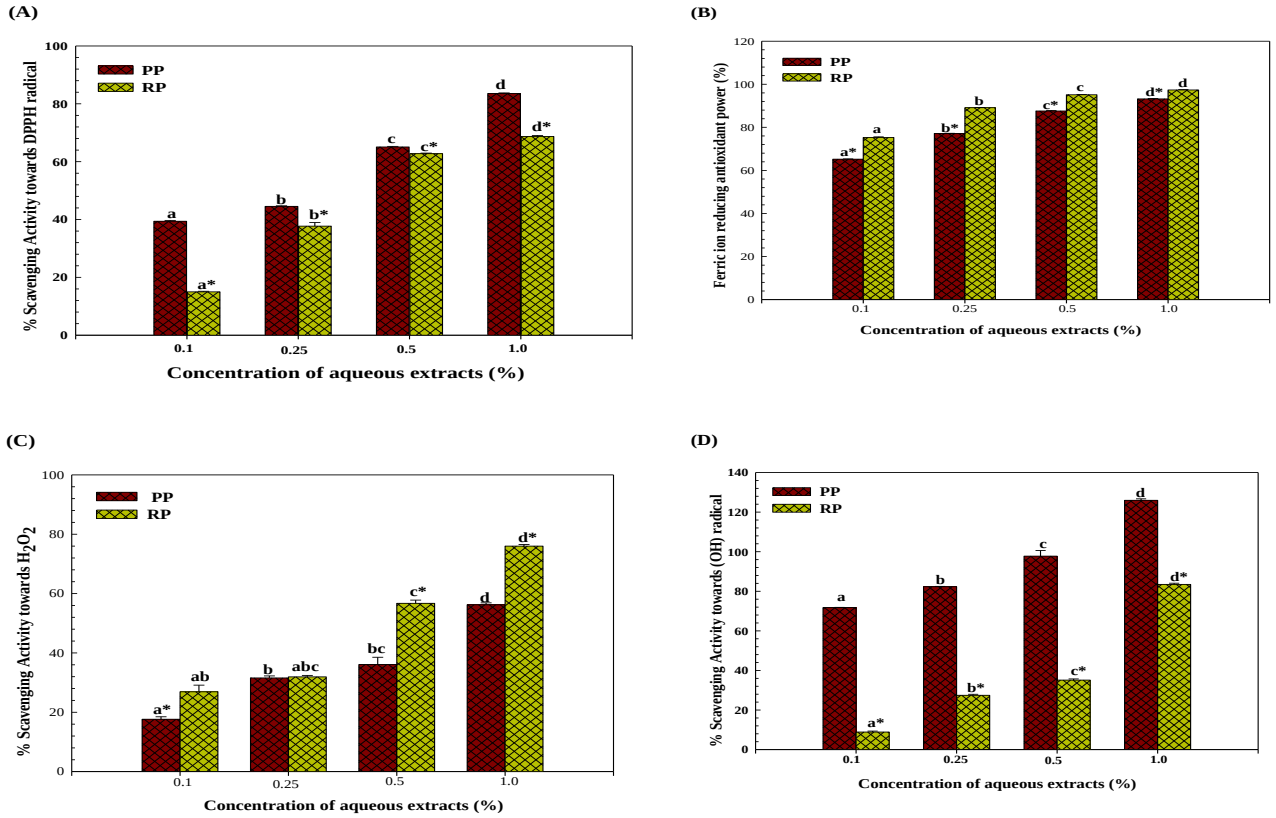

Figure 2: Effect of hydro-distillation on scavenging activity of DPPH (A), FRAP (B), H_2O_2 (C), ·OH (D) of *H. suaveolens* aqueous extracts prepared from plant powder (PP) and residue powder (RP). Data represents the significance difference between RP and PP applying 2 samples t– test at $P \leq 0.05$. Different alphabetical letters represented for each concentration of water extracts represents significance at $P \leq 0.05$.Bars represent standard error.

Further, a correlation analysis was carried out between TPC and TFC of PP and RP with their percent scavenging activity of hydrogen peroxide **(Table. 1).** It shows a strong correlation between TPC and TFC of RP with their percent scavenging activity i.e. r=0.984 and r=0.995, respectively. In case of PP, it also showed the significant correlation between TPC and TFC and percent scavenging activity i.e. r=0.970 and r=0.958, respectively **(Table. 1)** which also showed a strong correlation with its scavenging ability.

3.5. Ferric ion reducing antioxidant power (FRAP)

Antioxidative activity has been proposed to be related to reducing power. Antioxidants can be explained as reductants and inactivators of ROS [46].The antioxidant activity of natural products is correlated with their reducing powers [47] for example, conversion of Fe^{3+} to Fe^{2+} ions. Reducing power in turns reflects electron donating capacity of bioactive compounds. In this assay, the presence of antioxidants in test samples resulted in the reduction of the $Fe^{3+/}$ferricyanide complex to the ferrous form by donating an electron. The Fe^{2+} was then monitored by measuring the formation of Perl's Prussian blue [36]. Increasing percent scavenging (%) of the

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reaction mixture indicates an increase in reducing ability. The ferric reducing ability of aqueous extracts reducing the ability of PP showed better percent scavenging ability as compared to RP **(Fig. 2B).** A correlation analysis was carried out between TPC and TFC of PP and RP and their percent scavenging ability **(Table 1).** It showed good correlation between TPC with PP (r=0.830) and RP (r=0.936). TFC also shows a positive relationship between TFC and PP (r=0.840) and RP (r=0.890).

3.6. DPPH scavenging activity

Free radical scavenging is one of the known mechanisms by which antioxidants inhibit lipid oxidation. The method of scavenging DPPH free radicals can be used to evaluate the antioxidant activity of specific compounds or extracts in a short time. DPPH is a stable nitrogen-centered free radical. Its colour changes from violet to yellow upon reduction by the process of hydrogen-or electron- donation. The model of scavenging the stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of various samples [48]. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers [49]. It was found that the radical scavenging activity of *H. suaveolens* aqueous extracts (PP and RP) was increased with increasing concentration of extract in a dose dependent manner. The DPPH radical scavenging activity of PP was more than RP at all concentrations **(Fig. 2A).** Besides this, a correlation analysis was carried out between TPC and TFC of PP and RP with their percent scavenging activity **(Table 1).** The percent scavenging activity of both the cases i.e. PP (r=0.98) and RP(r=0.86) was strongly correlated with the TPC. Also in case of TFC, it indicates a positive relationship between these two i.e. PP (r=0.964) and RP (r=0.855). From the results, it was clear that in both the cases i.e. PP and RP it showed a very strong relationship between the DPPH scavenging activity and their TPC and TFC.

Table 1: Pearson's correlation coefficients (*r*) values between different antioxidant assays and total phenolic content (TPC) and total flavonoid content (TFC) of plant powder (PP) and residue powder (RP) aqueous extracts.

		Antioxidant assays				
	Extract	**DPPH**	**FRAP**	**·OH**	**H_2O_2**	**TAA**
TPC	**PP**	0.98	0.83	0.97	0.97	0.99
	RP	0.86	0.94	0.99	0.98	0.99
TFC	**PP**	0.96	0.84	0.98	0.96	0.98
	RP	0.85	0.90	0.94	0.99	0.98

3.7. Relationship between total phenolic (TPC) and flavonoid content (TFC) with various antioxidant assays

The results showed that the total phenolic and flavonoid content of PP and RP of *H. suaveolens* were strongly correlated with various antioxidant assays (TAA, H_2O_2 and ·OH) and correlation coefficient values (*r*) was represented in **Table 1**. The differences in correlation coefficient among different antioxidant methods indicate the fact that single assay may not be used to assess the total antioxidant activity [50]. Similar to our study, some authors have also reported linear correlations between total phenolic content (TPC) and antioxidant assays [51, 52]. The results suggested that the phenolic and flavonoid contents contributed significantly to the antioxidant capacities of aqueous extracts of *H. suaveolens*. Similar relationships have been widely reported in many plants

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[53, 54, 55]. Both phenolics and flavonoids are responsible for antioxidant activity of plant extracts [56]. A few other studies have also shown that waste residues of plant material could be utilized for antioxidant properties [57]. It is thus clear that *Hyptis* plant extracts possess total antioxidant activity that could be utilized in many ways. Further, antioxidant activity was also high in waste material (RP) left after oil extraction bear great significance owing to the possible utilization of waste resource.

IV. CONCLUSION

At last, it was concluded from the present study, that *H. suaveolens* produces a nonprofitable waste and this by-product generated during hydro-distillation process could be valorized and used as a source of natural antioxidants. The present is the stepping stone towards various other industrial processing of phyto-pharmaceuticals, food products and plant-based especially essential oil industries where after extracting the essential oils large amounts of organic waste are produced every year. These organic waste products are disposed as waste material; however, it still contains several valuable compounds (e.g. antioxidants) therefore, it should be further utilized to produce high value added products with a specific efficacy.

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