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ANTI-OXIDANT ACTIVITY EVALUATION OF DIFFERENT PARTS OF CHAMOMILE (*MARTICARIA CHAMOMILLA L.*) EXTRACTS

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ABSTRACT

The antioxidant and antimicrobial activity of chamomile was evaluated by preparing different extracts from different parts (stem, root and flower) of chamomile plant by applying different test system of three verities. Various extracts prepared from three different parts (stem root and flower) of chamomile by using 2 solvents (ethanol, ethanol: water and water). The antioxidant activity was performed using different biochemical assays like- 2,2-diphenyl-1-picrylhydrazine (DPPH), Nitric oxide (NO), ferric reducing assay (FRAP), Reducing power assay (RP), Total antioxidant capacity (TAC), Total flavonoids determination (TFD) and Total phenolic determination (TPD). The three extract PRW, VSW and SFH showed higher flavonoid content compared to other tested extracts. SFH, PSE, PFH, PSW VFH and VSH showed higher reducing potential in comparison to other extracts at a tested concentration of 100µg/ml.

Keywords: Chamomile, Extracts, Antioxidant Activity, Marticaria Chamomilla

INTRODUCTION

The word chamomile name is taken from the Greek word "Khamai" which means "On the ground" and melon, which means, 'Apple'. The chamomile plant is also called Matricaria chamomilla L. it is an important herb which has various medicinal properties, and this plant is originated from the south and east Europe. Matricaria chamomilla is also found in various countries including Brazil, France Hungary, and Germany. It is introduced in India from ancient time and currently, it is grown in Jammu & Kashmir, Maharashtra, and Punjab. The varieties of plants may be seen in Asia, Australia, New Zealand, North Africa, and in North America also (Ivens, 1979). Among these Hungary is the largest maker of the plant biomass. The cultivation of the plant in India is held in the Lucknow for about 200 years and also in Punjab for about 300 years. In India the plant was first propagated in the Lucknow in the alkali soil (Handa et al., 1957).

The plant chamomile used as the herbal medicines from ancient time and the 26 countries mentioned in their pharmacopeia that it is used in various drugs.

It is an important plant constituent of various Unani and Homeopathic medicines preparation (Mann & Staba., 2002). The Chamomile's flower contains the vital oil (Blue) 0.2-1.9% which includes lots of uses (Bradley, 1992).Matricaria chamomilla is commonly used as an anti-inflammatory, antiseptic, antispasmodic and mildly sudorific. This is similarly used internally for the disturbance of stomach which includes pain, indigestion, loose motion and nausea. This is not more effective for painful menstruation cycle and the inflamed urinary tract. The

chamomile powder used for the wound healing, skin irritation, infection like- shingles and boils and also used for hemorrhoids, inflammation of the mouth, throat and eyes (Fluck, 1988).

Matricaria chamomilla includes other pharmacological properties like (Carminative, healing, sedative and spasmolytic activity (Salamon, 1992). Chamomile has the antibacterial activity against both the gram negative and gram positive bacteria. Its oil used for mild sedative, antibacterial, fungicidal action. Chamomile is in the perfumery, cosmetics, aroma therapy and also in food industry (Lal et al., 1993).The demand of chamomile flowers is very high because it widely used as the herbal tea, treatment of cough and cold and also in baby massage (Anonymous, 1969).

MATERIAL AND METHODS

Source of plant material

The chamomile plant parts (Flower, Stem, and Root) were collected from CSIR-Central Institute of Medicinal And Aromatic Plant, Lucknow-226015, Uttar Pradesh, India. The collected plant material was identified authenticated by Botany and Pharmacognosy Department of CSIR-CIMAP, Lucknow. The plant parts were allowed to shade dry and crush them in a mechanical grinder.

Preparation of extracts

The fresh plant parts (flower, stem, and root) were collected and the fresh weight was recorded. The plant materials were shade dried and the weight of dried material were recorded. The plant material (flower, stem, and root) was dipped into the solvents (Ethanol, Ethanol: water, Water). The process was repeated at least 3 times with each solvent (Ethanol, Ethanol: water, water). The plant extract was collected and the solvent was removed by using vacuum Rotavapor. Collect the final extract of plant and the weight was recorded.

Extraction

The process used for extraction was percolation and have been divided into three steps.

Percolation

Percolation is the method by which the plant materials are filled in separating funnel and the particular solvents are poured and the plant materials are fully dipped with the solvent. After standing for about 24 h, the menstrum is collected in a container at a speed of 2-4 drops/seconds without pressing the marc. The menstrum collected was further process to concentrate the extract(s).The same cycle was repeated with all those solvents used in the process.

Concentration

In this step, the collected menstrum was taken to concentrate using an instrument called 'Rotavapor'. For concentrating the extract, the menstrum was taken in the round bottom flask (RBF) which is rotated at a regular speed of about 3-4 cycles in a second with the help of automatic motor and the filtrate at temperature 40-500C was concentrated under reduced pressure. This step consists to facilitate the dried matter for further steps, using for instance evaporation.

Final extract

For final extract(s) preparation, the air drying process was used at room temperature. After drying the final extract(s) obtained were put in vials, depending on the extract(s) amount and stored at 40C for further testing.

ANTIOXIDANT ACTIVITY

The antioxidant assays, in the present study were performed in a concentration-dependent manner, i.e. the response (O.D) of all the extracts samples were either decreasing or increasing with increase in their respective concentration.

Chemicals

DPPH dye, methanol, Tris HCL, Sodium nitroprusside, PBS solution, Sulphanilamide, phosphoric acid, NED dye, Disodium hydrogen phosphate, sodium dihydrogen phosphate, TCA, Potassium ferricyanide, ferric chloride, FRAP reagent, acetate buffer, TPTZ, folin's reagent, sodium carbonate, sodium phosphate monobasic, ammonium molybdate, H_2SO_4, aluminium chloride, and Potassium acetate were obtained from CIMAP Lucknow.

Procedure

DPPH Assay

The DPPH radical scavenging activity of plant extract was measured according to the method of) (Luqman et al.,2012).An aliquot of stock solutions from plant extract was prepared in a concentration of 10 mg/mL and 1mg/mL which was serially dilute to working concentration (0.8µg, 2µg, 4µg, 10µg). Then add100µL of methanol followed by the addition of 400µL TrisHCL buffer (100mM, PH-7.4).In the next step, 500µL of DPPH (0.5mM), was added to the aliquots. The samples were mixed thoroughly and left at 370C for 20 min in incubator. The absorbance of extracts was taken at 517 nm. All the experiments were performed using 96-well cell culture plates in replicate.

The capabilities to scavenge DPPH radicals were calculated via following formula:

$$\% \text{ Scavenging Potential (DPPH)} = \frac{[\text{Absorbance of control} - \text{Absorbance of sample}] \times 100}{(\text{Absorbance of control})}$$

NO Assay

This method was performed as per the method of (Ahmed et al., 2014). Sodium nitroprusside in the solution of PBS at pH 7.4 is responsible for the generation of free radicals (NO) which after interaction with oxygen produces nitric oxide .In the assay, plant extract sample were mixed with 200µL sodium nitroprusside which was formerly prepared via using PBS in distilled water. Then, the samples were incubated at the 25-300C temperature for 30 min. After incubation, from all testing solutions, 50µL of the incubated solution was mixed with 100µL Griess reagent. In the final step, the absorbance of all the samples was read at 546 nm against the corresponding blank solution.

The capacity to scavenge NO ions can be measured via the following formula:

$$\% \text{ Scavenging Potential (NO)} = \frac{[\text{Absorbance of control} - \text{Absorbance of sample}] \times 100}{(\text{Absorbance of control})}$$

RP Assay

This assay used the previous reported protocol of (Maurya et al., 2017).In the first step, the different concentration of plant extract was mixed with 250µL of phosphate buffer (0.2M, pH 6.6).250µL of 1% potassium ferricyanide was added to all the micro-centrifuge tubes of samples and mixed thoroughly. Then, it was incubated for 20 min at 500C.After the incubation, 250µL of 10% TCA was added and centrifuged at 5000 rpm for 5 min.125µL of supernatant from the mixture was taken and mixed with 125µL of distilled water and 25µL of freshly prepared ferric chloride. After thoroughly mixing the contents. The absorbance of all samples was measured at 700 nm. All the samples were taken in the replicate using 96-well plates.

FRAP Assay

FRAP assay for different extracts, sample was carried out by the method of (Noreen et al. 2017). Different concentration of extracts was added in 50µL of distilled water. Then, 1.5 mL FRAP reagent was added to the

tubes and mixed thoroughly. The mixture was incubated at 370C for 5 min. The absorbance was recorded at 593 nm. All the tests were performed using 96-well cell plates in replicate.

Total Phenolic Content Estimation

For the estimation of total phenols in the compounds, Folin-Ciocalteu reagent method was used (Luqman et al., 2012). An aliquot of different concentrations of plant samples were mixed in 50µL of distilled water. In the next step, 250µL of folin's reagent was added and mixed thoroughly. Then, 7.5% Sodium carbonate was added to all the aliquots and the incubation period was for 90 min at 370C.All the test extracts were measured via taking absorbance at 765 nm. All the determinations were performed in replicate forms using 96-well cell culture plates.

Total Flavonoid Content determination

The total flavonoids content of plant extract samples was determined using the protocol of (Maurya et al., 2017).In this assay, the different concentrations of all the samples were mixed with 50µL of distilled water. Then, 150µL methanol (1M) and 10% 10µL Aluminium chloride were mixed thoroughly into the tubes followed by the addition of10µL potassium acetate (1M) and 280µL distilled water. All the tests sample were incubated at the temperature (25-370C) for 30 min and the absorbance was taken at 415 nm using UV-Visible Spectrophotometer.

Total Antioxidant Capacity determination

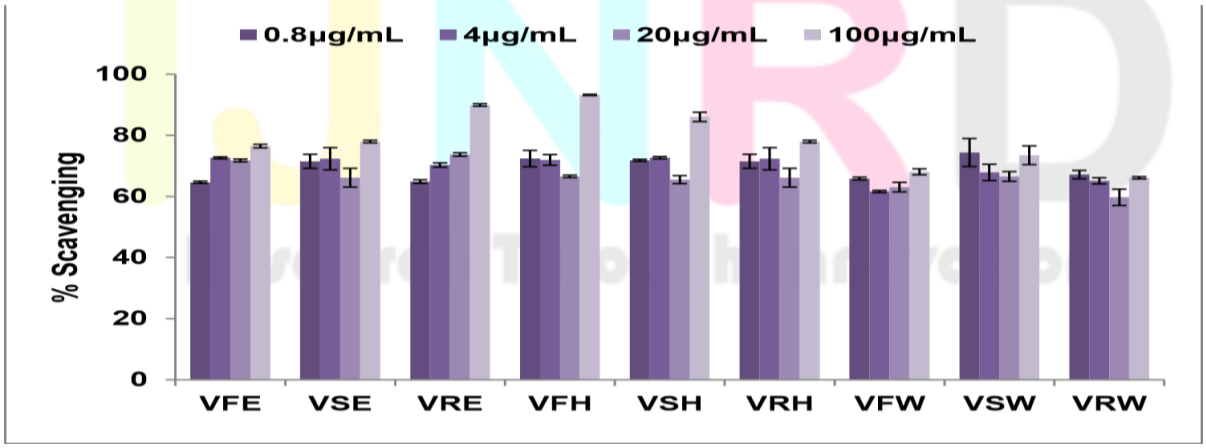
The method for this assay implies the standard procedure of (Nooren et al., 2017).In this method, different concentration of plant extracts were taken in 1.5-2 mL micro-centrifuge tubes and were mixed with 1mL reagent solution (30mM Sodium phosphate, 4mM Ammonium molybdate and 1M conc. H2SO4).Then, these tubes of sample were incubated at 950C for 90 min. The corresponding blank solution was also taken in a tube along with different samples. The absorbance of all the compounds was recorded at 695 nm.

Results and Discussion

Antioxidant activity

DPPH Assay

The DPPH radical scavenging assay was performed, in this assay antioxidants react with DPPH and reduce it to the yellow colored diphenyl-picry-lhydrazine. The experimental observation of scavenging effect of three varieties of chamomile extracts was showed in figure 8. In vitroanalysis reveal that VRE,VFH and VSH were better scavenger (>80% scavenging) than other extracts in vallery extracts .whereas PFE ,PFH, PSH and SSE,SFH SFW showed higher % scavenging (>80%) in prashant and sammohak varieties extracts.



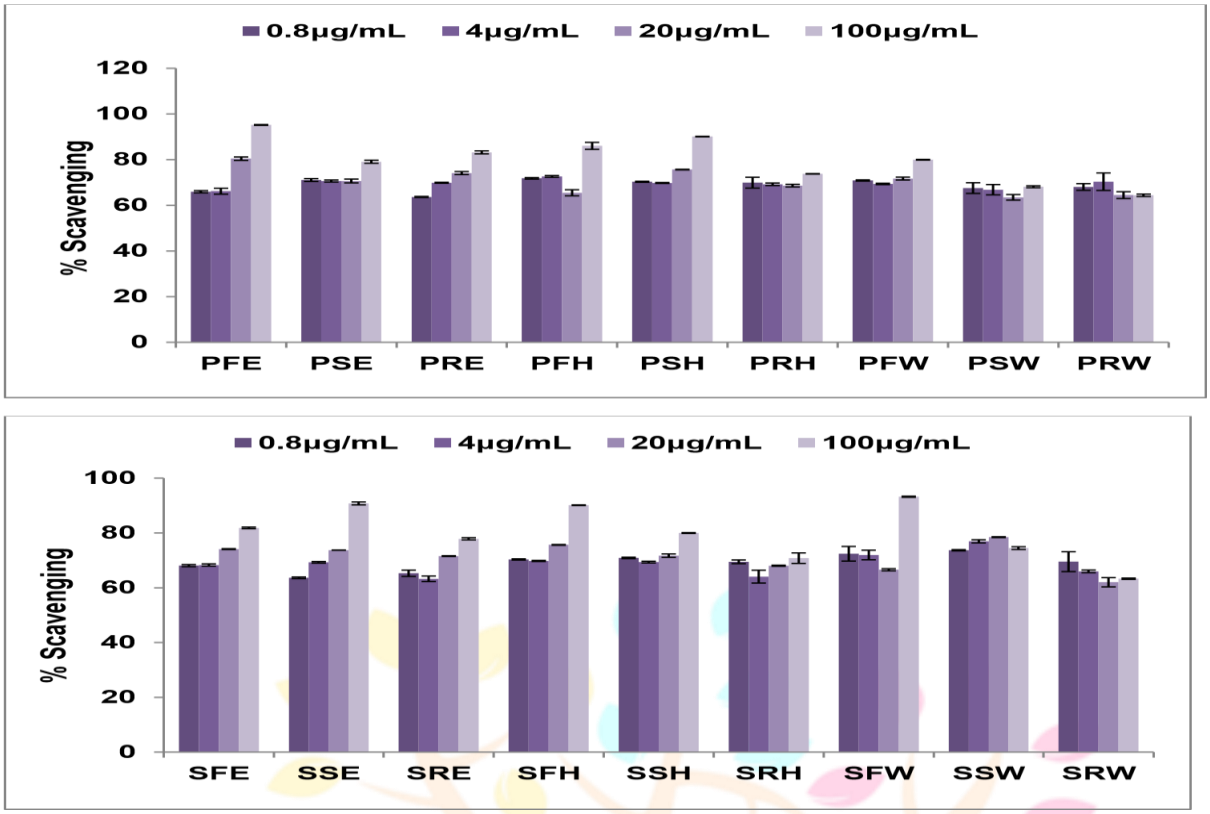
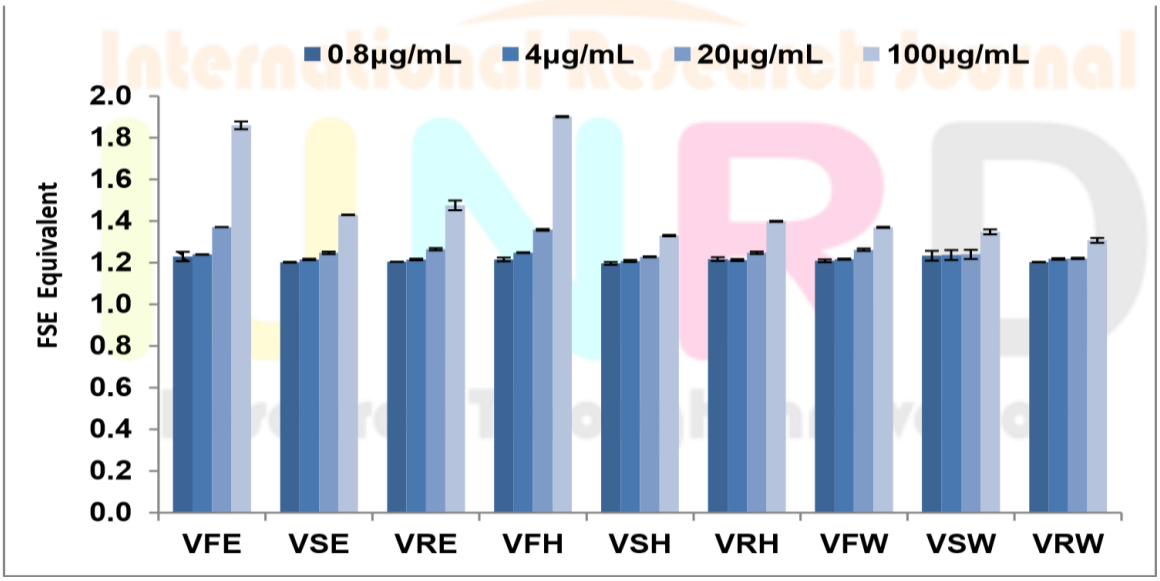


Fig 1.: DPPH Scavenging activity of chamomile extracts in different concentration

Ferric reducing Power assay

The Ferric reducing antioxidant power assay was used to figure out the antioxidant capacity of different extracts of all three varieties of chamomile. In this assay results showed that the antioxidant activities were highest for VFE ,VFH in Vallery extracts, PFE, PRE,PFH and PSH in present variety extracts and SSE , SFH in sammohak variety extracts.



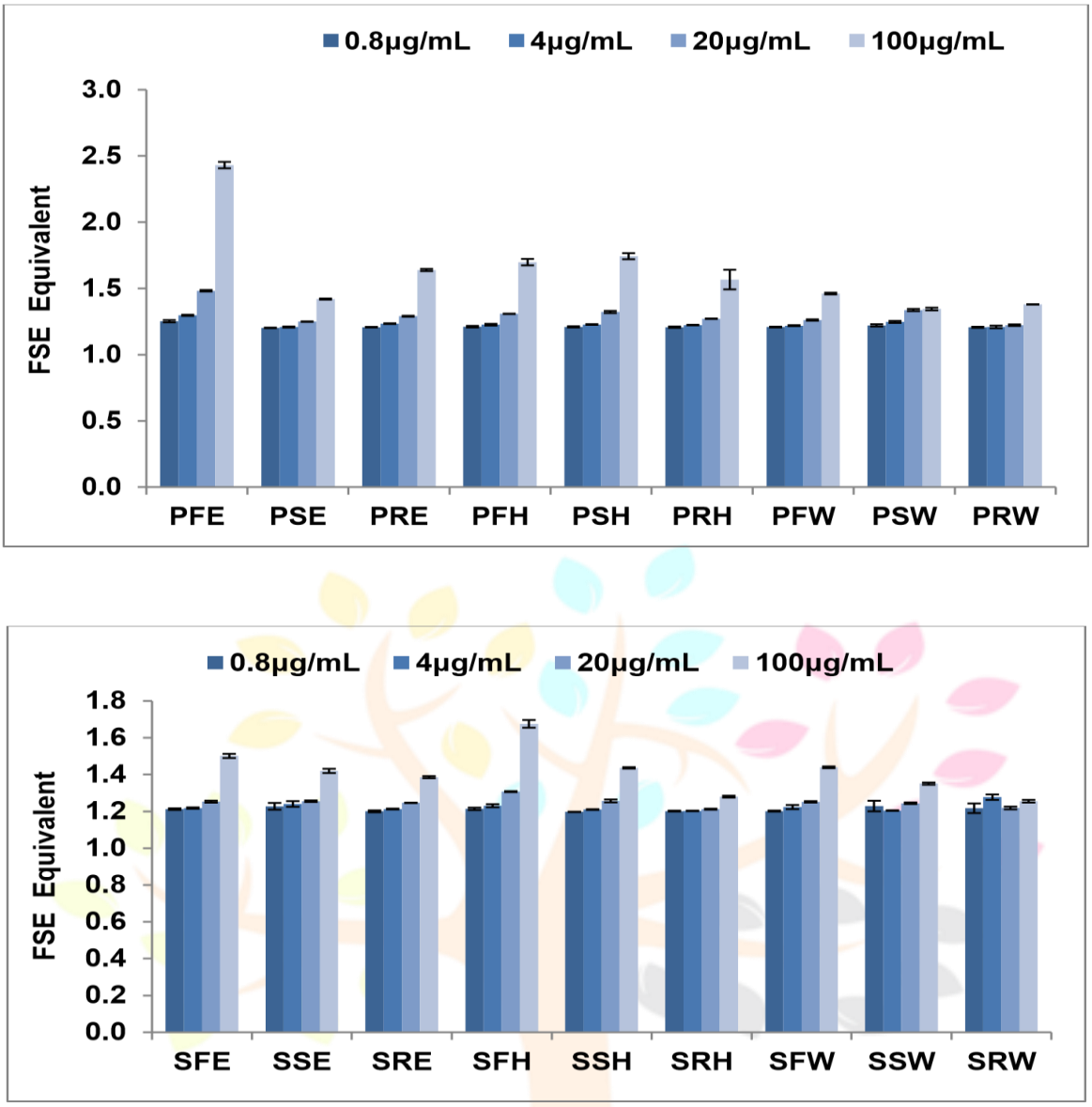
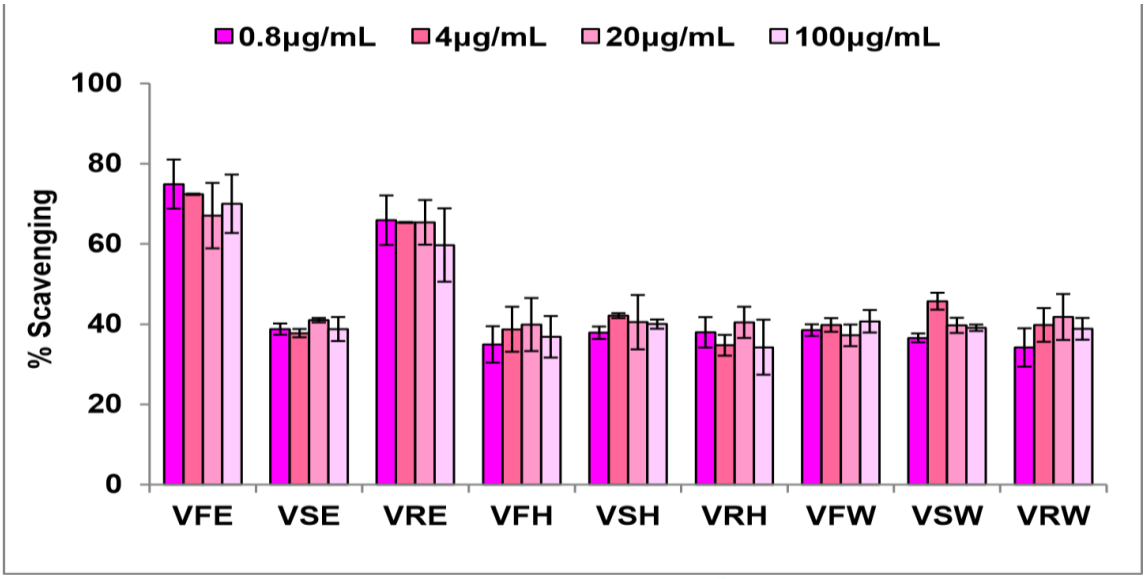
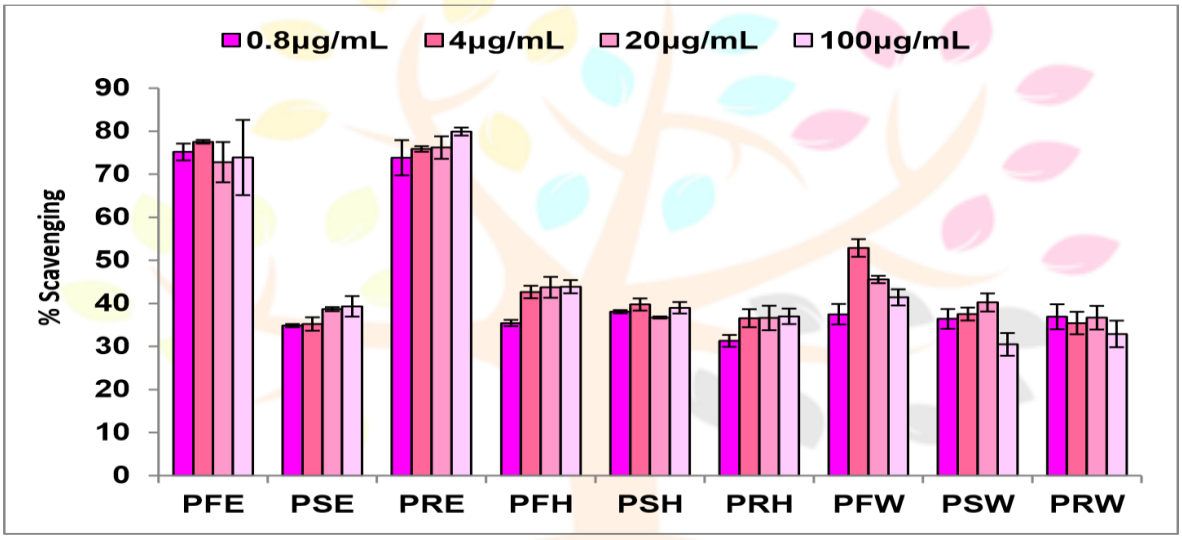


Fig 2.: ferric reducing activity assay of chamomile extract in different concentration

NO scavenging assay

The NO scavenging capacity was determined by a decrease in the absorbance at 546 nm, induced by antioxidants. The altered change of optical density of NO was supervised to evaluate the antioxidant potency through NO scavenging by the test samples. The results of NO scavenging activity of different extracts are shown as percent of NO scavenging. It has been noted that VFE, VRE, PFE, PRE, SSE, SRE and SSW have a greater inhibition (≥80%) comparative to other plant extracts with respect to varieties.





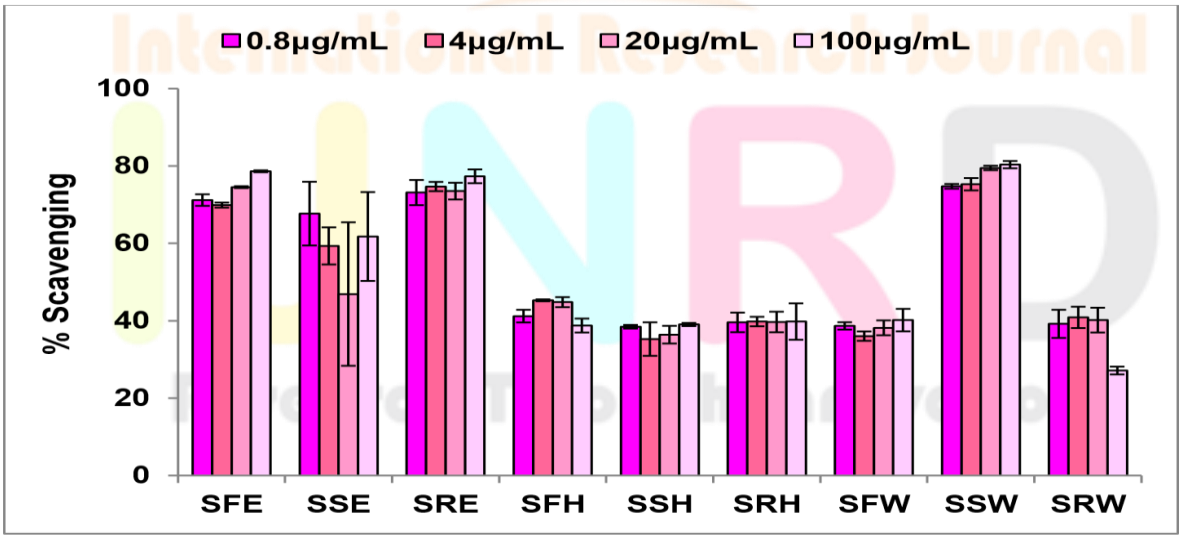
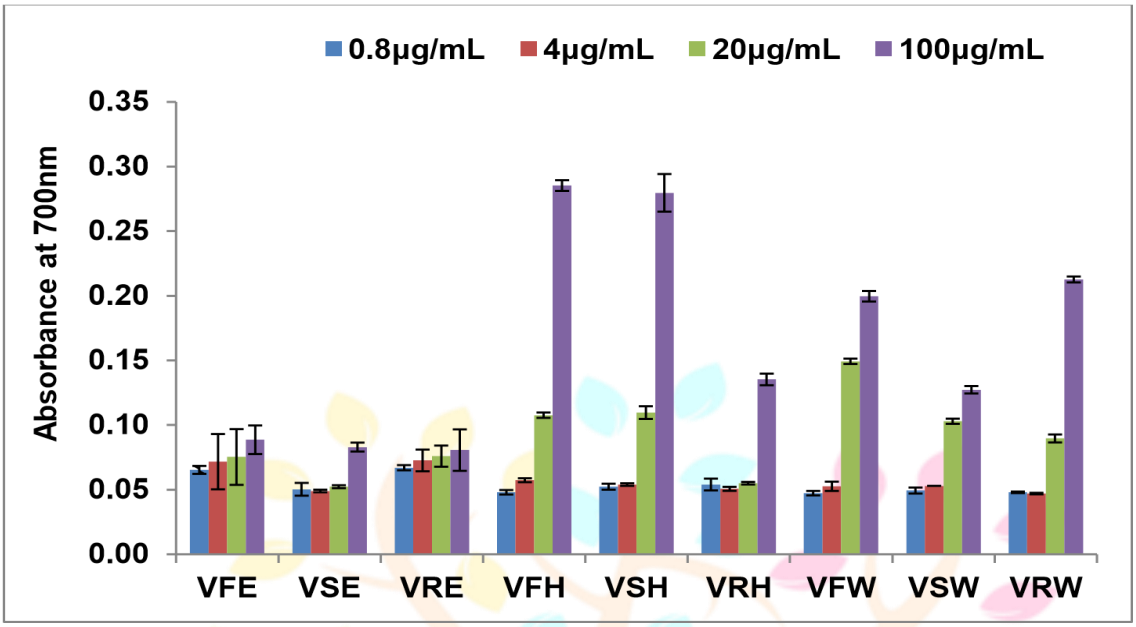


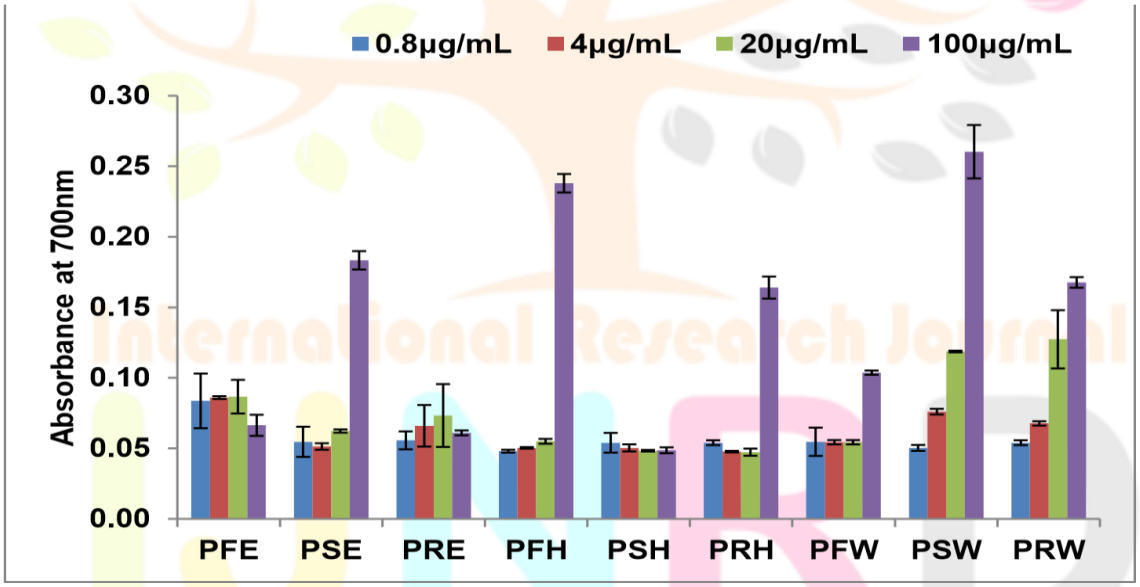
Fig 3.: NO scavenging activity assay of chamomile extract in different concentration

Reducing power assay

The Reducing power of different solvent extracts of all three varieties of chamomile was determined and the results are shown in Figure 11.The VFH VSH VFW and VRW extracts of vallery varieties , while PFE,PFH,PRH,PSW and PRW extracts of prashant variety and SFH ,SRW extracts of sammohak variety displayed the highest reducing power. All of the above extracts showed almost similar increasing trend in reducing

power with the increase in extract concentration. In this assay,antioxidants act as reducing agent and reduced the Fe3+/ferricyanide complex to the ferrous form. Therefore, measuring the formation of pearl Prussian blue at 700 nm can monitor the Fe2+ concentration.





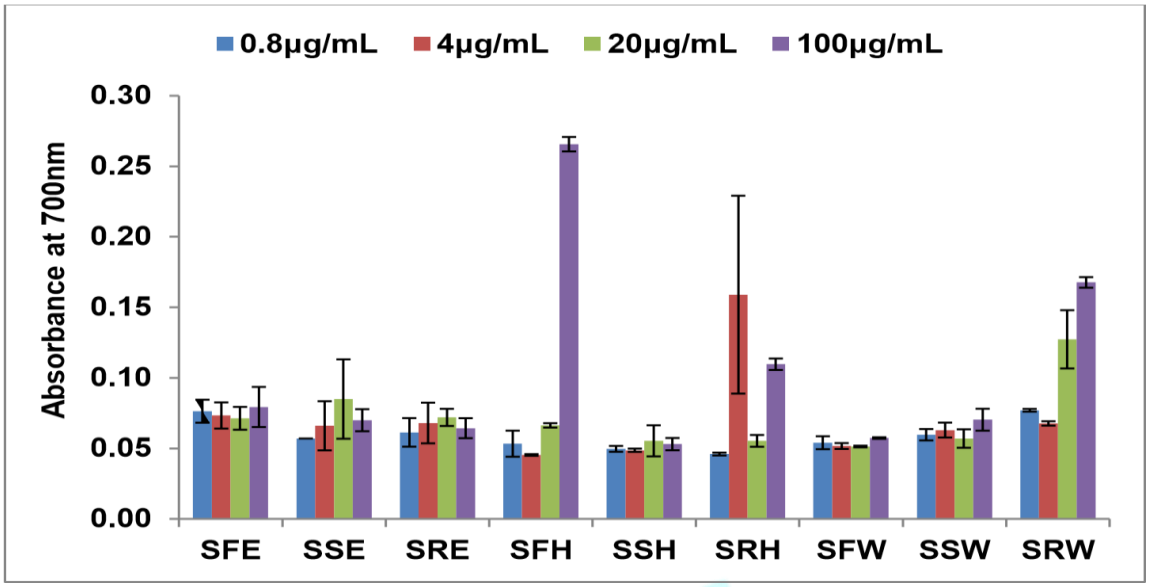
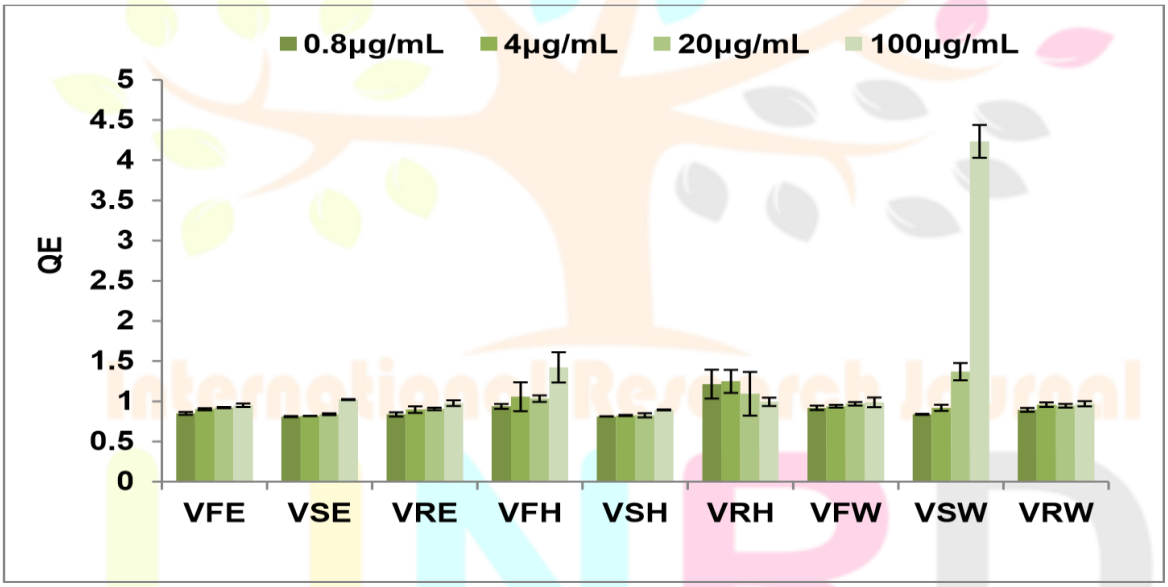


Fig 4.: Reducing power activity of chamomile extract in different concentration

Total flavonoid content

The total flavonoids content of the different extracts were determined in terms of µg of quercetin equivalents and the results are shown in Figure 12 depicted that VSW, PRW and SFH extract had the highest total flavonoids content compared to other extracts respective to their varieties.



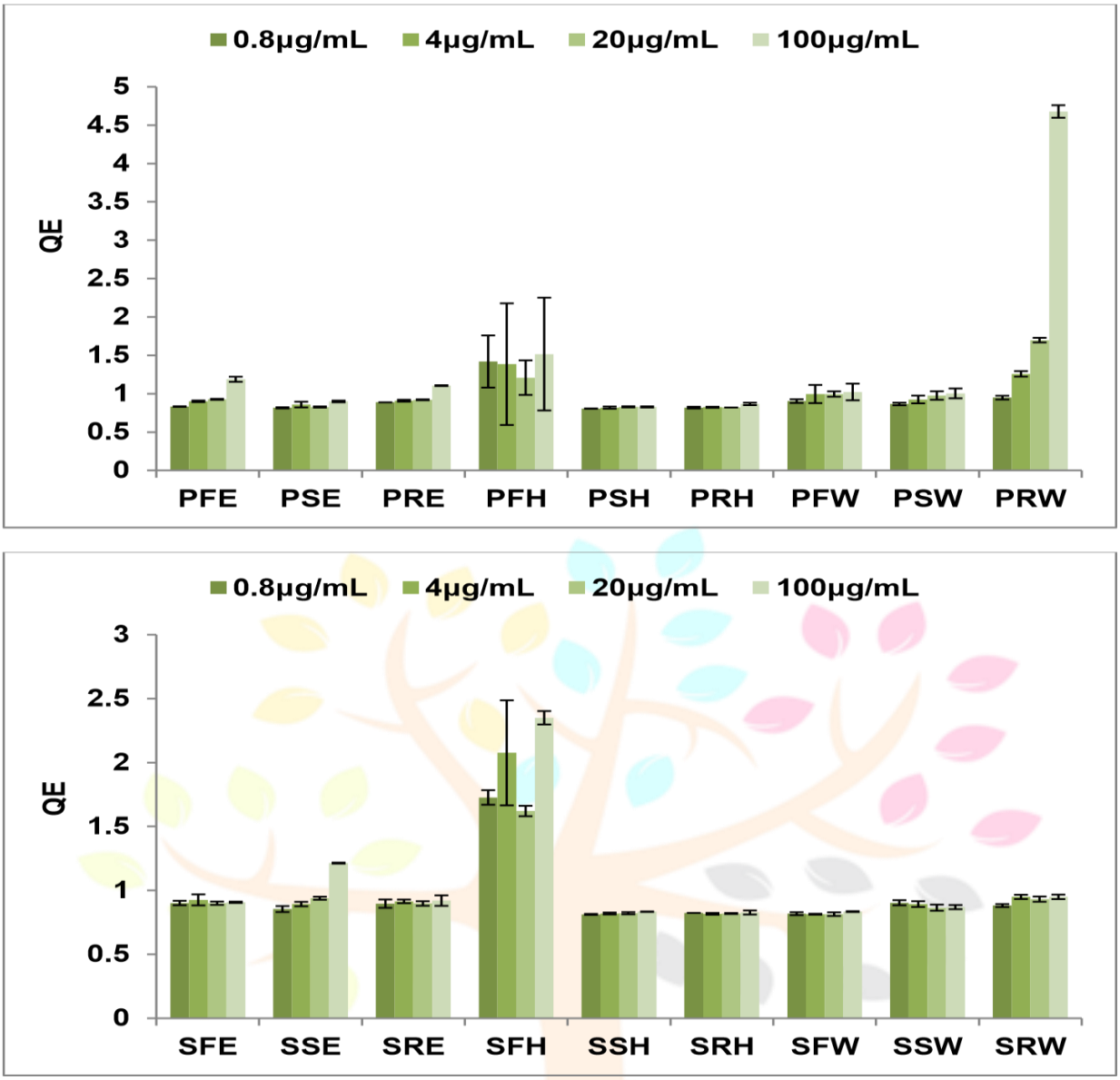
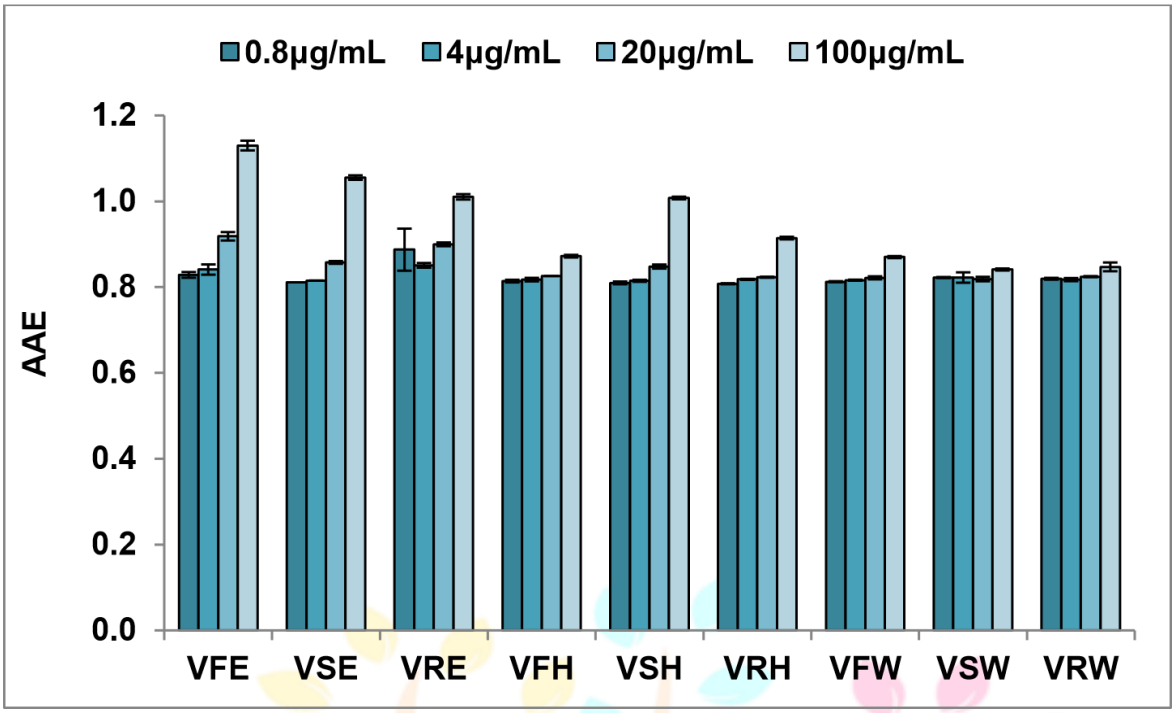


Fig 5.: Total flavonoids content determination of chamomile extract in different concentration

Total antioxidant capacity

The total antioxidant capacity of the extracts were determined in terms of µg of ascorbic acid equivalence and the results of this test suggest that VFE,VSE,VRE,VSH,PFE,PFE, SFE,SSE and SRE exhibit higher antioxidant capacity.


0.8µg/mL
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VFE
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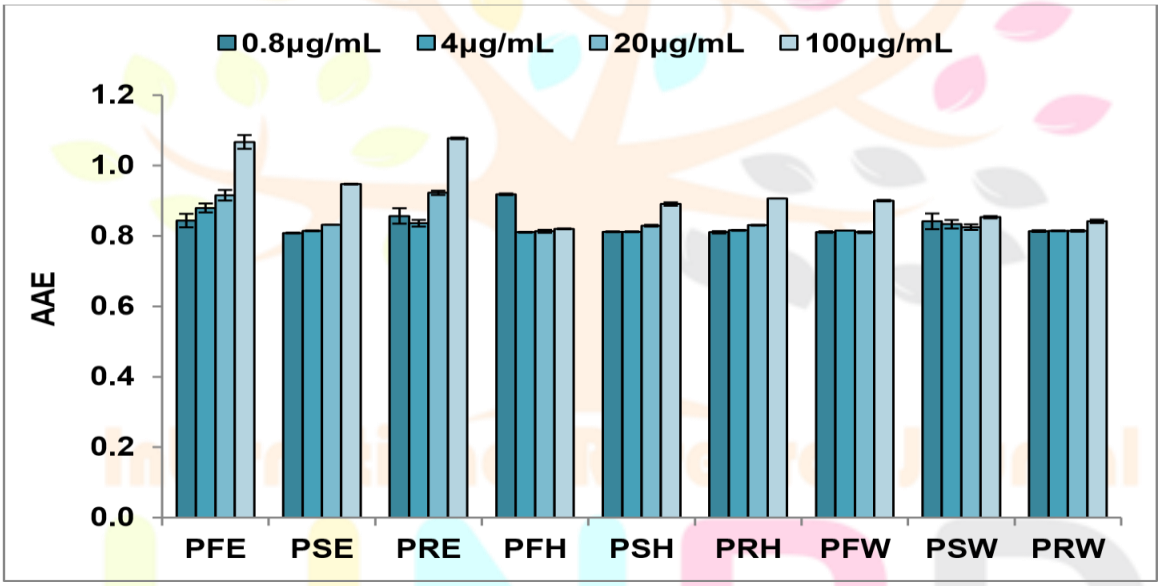
VRW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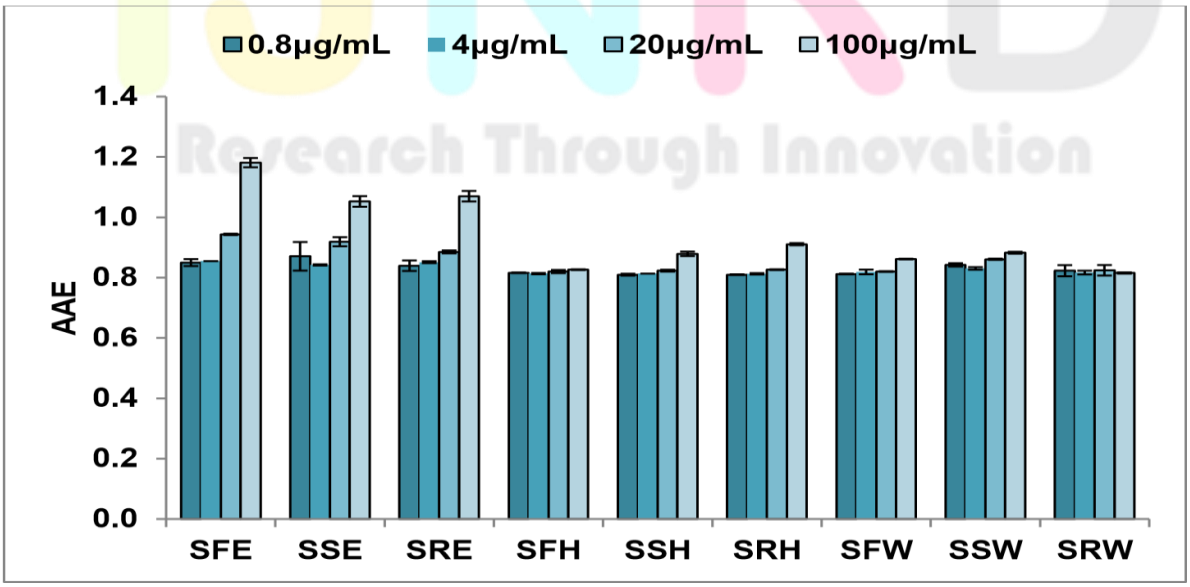
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0.0
SFE
SSE
SRE
SFH
SSH
SRH
SFW
SSW
SRW

Fig 6.; Total antioxidant capacity of chamomile extract in different concentration

CONCLUSION

The present investigation was designed to prepare the extracts of different parts of plants including stem, root and flower using ethanol, water and ethanol: water (50:50). The three varieties of chamomile were selected and extracts were prepared. The extracts were tested for the antioxidant activity. In antioxidant assay, seven different biochemical assays were performed to evaluate the antioxidant potential of prepared extracts. The extracts showed strong free radical scavenging potential at all the tested concentration via inhibiting the formation of DPPH and NO radicals. As we know that the free radicals formation is the main cause of various stress associated diseases/ disorders. The available treatments also have a number of side effects. Therefore, further some active leads are required from medicinal plants to reduce the burden. Because chamomile extracts showed strong antioxidant potential in the tested biochemical assays so it can be further explored for the isolation and characterization of active principles with their mechanism of action.

REFERENCES

1. Handa KL, Chopra IC, Abrol BK. Introduction of some of the important exotic aromatic plants in Jammu and Kashmir. Indian Perfumer. 1957; 1:42–9.
2. Mann C, Staba EJ. The chemistry, pharmacology and commercial formulations of chamomile. In: Craker LE, Simon JE, editors. Herbs, spices and medicinal plants- recent advances in botany, horticulture and pharmacology. USA: Haworth Press Inc. 2002; 235–80.
3. Bradley P. The British herbal compendium. In: Bradley P, editor. 1st ed. London: British Herbal Medicine Association; 1992.
4. Fluck H. 1st ed. London: W. Foulsham and Co. Ltd; 1988. Medicinal plants and authentic guide to natural remedies.
5. Salamon I. Chamomile a medicinal plant. J Herbs Spices Med Plants. 1992;10:1–4.
6. Lal RK, Sharma JR, Misra HO, Singh SP. Induced floral mutants and their productivity in German chamomile (Matricariarecutita) Indian J Agric Sci. 1993; 63:27–33.
7. Anonymous. Azulene in pharmacy and cosmetics. Dragoco Rep. 1969;16:23–5.
8. Luqman S Srivastava S Kumar R K,. Maurya, A K Chanda D. Experimental assessment of Moringa oleifera leaf and fruit for its antistress, antioxidant and scavenging potential using in vitro and in vivo assays, Evid. Based Complement. Alt Med. 2012;519084.
9. Ahmad A, Singh DK, Fatima K, et al. New constituents from the roots of Oenotherabiennis and their free radical scavenging and ferric reducing activity. Industrial Crops and Products 2014; 58:125–132.
10. Maurya P, Singh S, Gupta MM, Luqman S. Characterization of bioactive constituents from the gum resin of gardenia lucida and its pharmacological potential. Biomedicine & Pharmacotherapy, 2017; 85:444–456.
11. Nooreen Z, Singh S, Singh DK, et al. Characterization and evaluation of bioactive polyphenolic constituents from Zanthoxylumarmatum DC., a traditionally used plant. Biomedicine & Pharmacotherapy 2017; 89:366–375.
12. Luqman S Srivastava S Kumar R K,. Maurya, A K Chanda D. Experimental assessment of Moringa oleifera leaf and fruit for its antistress, antioxidant and scavenging potential using in vitro and in vivo assays, Evid. Based Complement. Alt Med. 2012;519084.
13. Maurya P, Singh S, Gupta MM, Luqman S. Characterization of bioactive constituents from the gum resin of gardenia lucida and its pharmacological potential. Biomedicine & Pharmacotherapy, 2017; 85:444–456.
14. Nooreen Z, Singh S, Singh DK, et al. Characterization and evaluation of bioactive polyphenolic constituents from Zanthoxylumarmatum DC., a traditionally used plant. Biomedicine & Pharmacotherapy 2017; 89:366–375.