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Comparative Assessment of Anti-Oxidant Properties of Mango Seed Kernels of Neelam and Banganapalle Variety

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Abstract
Free radicals, the source of oxidative stress, have been linked to a wide range of disorders. Current researchisdirected towards finding naturally-occurring antioxidant of plant origin. The present investigation aimed to evaluate and compare the antioxidant capabilities of six solvent extracts of mango seed kernel (MSK) of two different mango species. The standard anti-oxidant assays have been done, including the Reducing power, DPPH, ABTS, Super oxide radical scavenging, Hydrogen peroxide scavenging, and Total anti-oxidant assays. Both the DPPH and ABTS assays show that MSK, after being extracted with ethyl acetate, has a high capacity to quench free radicals. Reducing power showed increase in absorption value withincreasing of concentration. Both the species have the same abilities to scavenge superoxide and hydrogen peroxide. Total antioxidant activity was high in the ethyl acetate solvent extract. In addition, our findings indicate that MSK extract may serve as a natural antioxidant.

Key words: Mango seed kernel (MSK), Free radicals, Oxidative stress, Anti-oxidants

I. Introduction

Mango, belongs to Anacardiaceae family and world's most popularly consumed fruits, is cultivated in many temperate and tropical regions. The fruit is commercially accessible as a dietary supplement because it is rich in nutrients necessary for human growth, development, and health. These nutrients include dietary fibre, lipids, proteins, carbohydrate, and phenolic compounds[1,2]. Epicarp (peel) and seed are the most common waste products from fruit processing. Three-fifths to two-thirds of a fruit's total mass is made up of its by-products. Annual production of the seed can exceed 75,000 tonnes, making it a potential source of pollution because there is no practicalway to dispose of it right now [3]. However, the mango seed kernels (MSK) withinthis agricultural waste product are seen as a significant source of biomolecules withcommercial potential.

The best mangoes come from India. It is estimated that India is potential productive centerof over a thousand distinct mango types. All the different kinds come in different colours, shapes, and flavours. *Neelam* and *Banganapalle* were the two types that are used for our research. Typically, smaller and with a tint of orange, *Neelam* Mangoes have a very pleasant aroma. Similarly, the *Banganapalle* type, commonly called as the king of mangoes, is rich in vitamin A and C[4].

Almost all processed foods nowadays are fortified with artificial antioxidants like butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tert-butylhydroquinone (TBHQ), etc[5,6]. However, use of synthetic antioxidants has been linked to negative health effects, which might be prevented with the use of natural antioxidants like those found in MSK[7,8]. This study has outlined the antioxidant qualities of the mango seed kernel of two different varieties. Thus, antioxidants derived from natural sources may serve as a viable substitute for manufactured substances. In addition, this research addresses the existence of many biomolecules in MSK.

The overarching idea of this work, which is situated within the framework of "green chemistry," is to suggest the use of substantial agro-industrial by-products, specifically the MSK of variety *Neelam and Banganapalle,* as a source of antioxidants in various sectors.

Even though numerous studies on antioxidant activity have been conducted, there are few reports on the assessment of the antioxidant activity of the mango variety (*Neelam* and *Banganapalle*). Considering this, the current study aims to evaluate the antioxidant activity of *Neelam* and *Banganapalle*. The results of this study may help identify a potential source of new therapeutic compounds for illnesses caused by stress, as well as their use as dietary supplements while receiving treatment and the development of new medications.

II. Materials and methods

II.I ***Collection and processing of kernel:***

The mango kernel was collected locally from Cuttack, Odisha is located between latitude 20.4958^0N and 85.9208^0E. The seeds were washed and air dried. The kernels and sheaths were removed manually from the seeds. Fresh kernel seeds were chopped into little pieces and dried at 50^0C for 2-3 days to remove moisture. The dry kernel was grinded and powdered.

*II.II****Extraction of plant material:***

In a Soxhlet system, powdered plant materials were extracted using n-hexane, chloroform, ethyl acetate, methanol, acetone, and distilled water. Then, a rotating evaporator removes surplus solvent. The residual solvents were vaporised by heating the extracts in an oven until they became semisolid.

II.III ***In-vitro anti-oxidant activity assay:***

*II.III.I****Reducing power assay:***Oyaizu (1986)

Oyaizu's method was used to test MSK solvent fractions for antioxidant capacity [9]. 1 ml of each solvent extract in 10% DMSO was combined with 2.5 ml of sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. After 20 minutes of incubation at 50^0 C, 2.5 ml of trichloroacetic acid was added and spun for 10 minutes. 2.5 ml of supernatant, 2.5 ml of distilled water, and 0.5 ml of ferric chloride [0.1% w/v in distilled water] were added, and absorbance was measured at 700 nm. 10% DMSO or water served as blank. Ascorbic acid are positive controls.

II.III.II ***DPPH assay:*** Chang *et al* (2001)

Using a modified Chang *et al.*,2001. method, the radical-scavenging activity of solvent extracts against DPPH was evaluated [10]. 1.5 ml of MSK extract in 10% DMSO was mixed with 1.5 ml of DPPH in

methanol. Control was DPPH and 10% DMSO in equal volume. The absorbance against methanol was measured at 517 nm after 30 minutes of incubation in the dark at room temperature. Ascorbic acid was the positive control. % Of DPPH inhibition was calculated using the following formula:

$$\%\ DPPH\ inhibition = [\{OD[control] - OD[sample]\}/OD[control]] \times 100 \qquad (1)$$

II.III.III ***ABTS assay:*** Re *et al* (1999)

In order to conduct the ABTS assay, we used the protocol established by Re *et al.*,1999[11]. An initial reaction volume of 2.7 ml was prepared by adding 0.3 ml of solvent extracts in 10% DMSO to the diluted ABTS. The control sample contained 0.30 millilitres of 10% DMSO and 2.70 millilitres of ABTS. The absorbance at 734 nm was measured after the sample was incubated for 60 minutes at room temperature and in the dark. To determine the percentage of inhibition, we used Equation (1). Butylated hydroxytoluene was used as positive control.

*II.III.IV****Superoxide radical scavenging activity:*** Patel *et al* (2012)

The Superoxide radical scavenging activity was determined by Patel *et al.,2012* [12]. Briefly, the 3ml reaction mixture consists of 0.01M phosphate buffer ($_p$H 7.8), 0.5mM EDTA, NBT (0.75mM), 20µg riboflavin and varying concentration of solvent extract.The absorbance at 560 nm was measured after the tubes were illuminated by fluorescent light for 6 minutes. As a control, methanol was employed. The use of ascorbic acid served as a positive control.

II.III.V ***Hydrogen peroxide scavenging activity:*** Ruch *et al* (1989)

Ruch *et al.*, 1989 [13] suggested a method for hydrogen peroxide scavenging. 1ml of various solvent extract strengths were combined with 40mM H_2O_2 and 2.4ml 1M phosphate buffer (pH 7.4). After 10 minutes of incubation, the absorbance was measured at 230nm. Blanks included phosphate buffer and H_2O_2. Positive control was ascorbic acid.

II.III.VI ***Total anti-oxidant activity:***Prieto *et al* (1999)

Prieto *et al.*, 1999 [14] evaluated total anti-oxidant activity. 4ml of phosphomolybdate reagent was added to 0.4 ml of MSK solvent extracts in 10% DMSO. Substituting 0.4 ml of 10% DMSO/distilled water for the solvent extract, the control sample achieved the same results. Aluminum foil was used to enclose the reaction mixtures, and they were incubated to 95^0C for 90 minutes. The absorbance was checked at 695 nm against a blank after cooling to ambient temperature. The blank was run in either water (for the ascorbic acid/aqueous extract) or DMSO (10%). MSK solvent fractions were used to calculate the ascorbic acid equivalent of the Mo [VI] reduction to Mo [V].

III. Results

In the current investigations, the anti-oxidant capacity of MSK of two varieties in different solvent extracts were determined and compared.

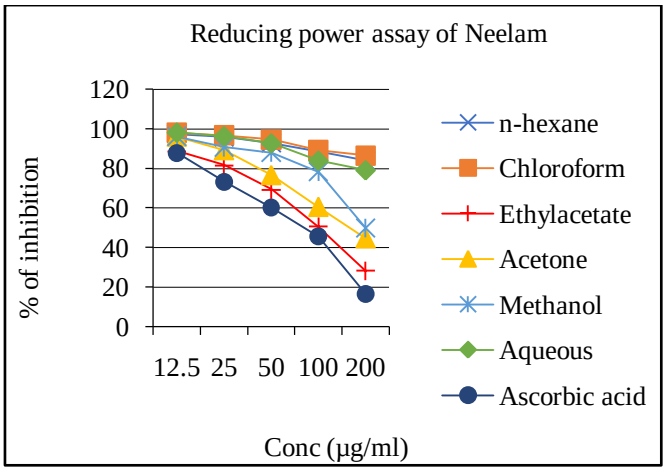

Figure 3 (a)

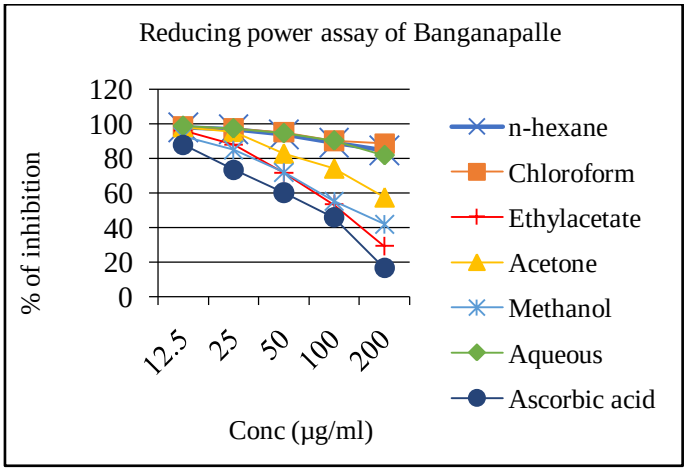

Figure 3 (b)

*III.I**Reducing power assay:***

Both figures above display results of the reducing power assay of two MSK variants obtained from distinct solvent extracts. Of all the substances tested, ethyl acetate at a concentration of about 200 µg/ml was found to have the highest reducing power. The IC 50 values for *Neelam* and *Banganapalle* were found to be 28.1348 ±0.1206 and 29.0078 ±0.0525 respectively. Ascorbic acid showed the IC 50 value of 16.5079 ±0.0715.

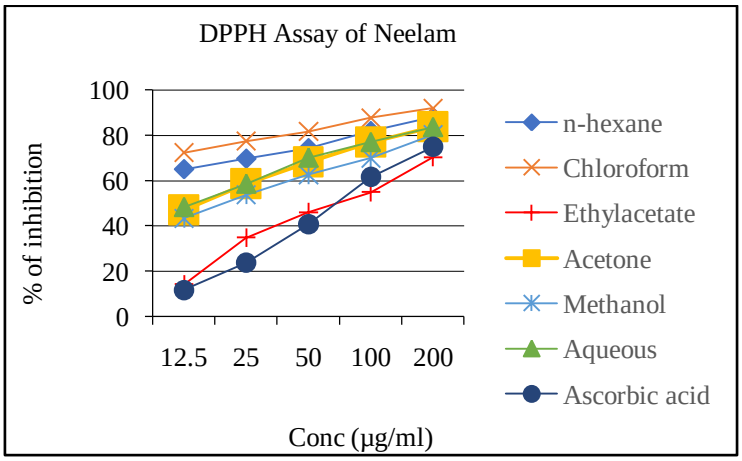

Figure 4 (a)

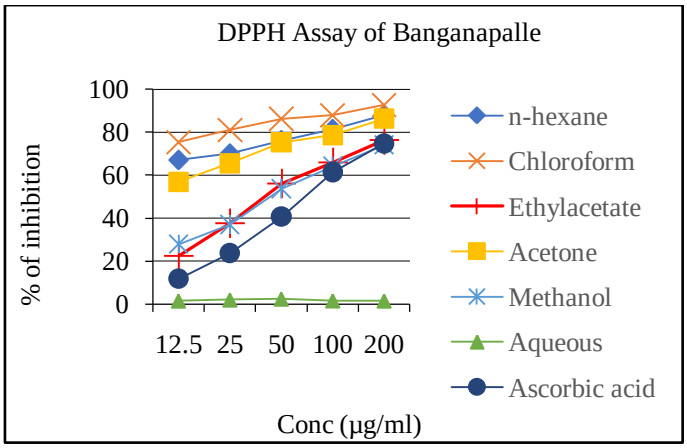

Figure 4 (b)

III.II ***DPPH assay:***

The above graph showed that in ethyl acetate extract, the IC50 value of 14.2156±0.8836 and 22.3039±1.2969 has been reported for *Neelam* and *Banaganapalle* respectively at 12.5µg/ml whereas at same concentration, ascorbic acid showed IC 50 value of 11.7647 ±0.4245.Again, Chloroform extract of both the varieties has shown IC 50 value of 91.9117±0.8490 and 92.647±0.4245 at 200 µg/ml concentration respectively.

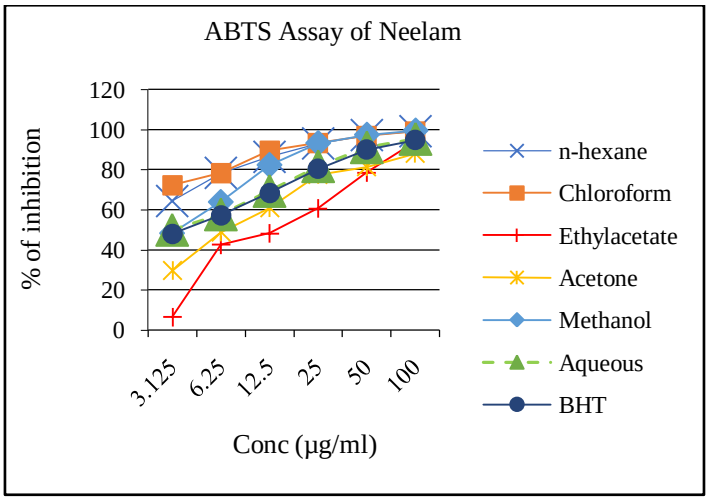

Figure 5 (a)

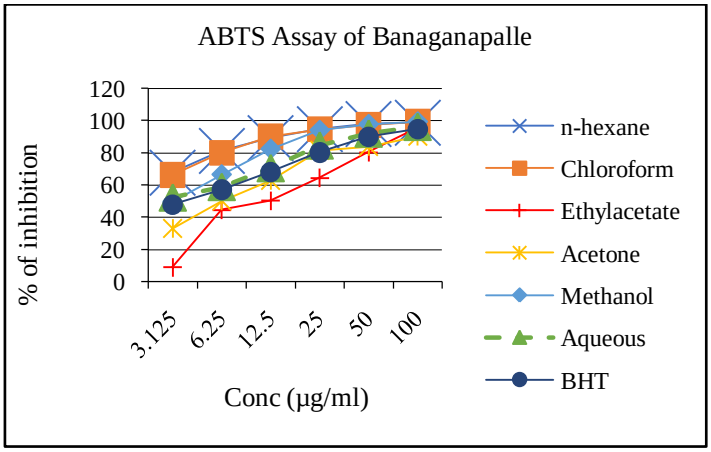

Figure 5 (b)

*III.III**ABTS assay:***

In the above graphs, the strongestanti-oxidant activity with an IC 50 value of 6.5704±0.1602 and 8.9743±0.5778 in ethyl acetate extract of Neelam and Banaganapalle at 3.125µg/ml concentration has observed while BHT showed IC 50 value of 47.9166±0.3205 in that concentration.

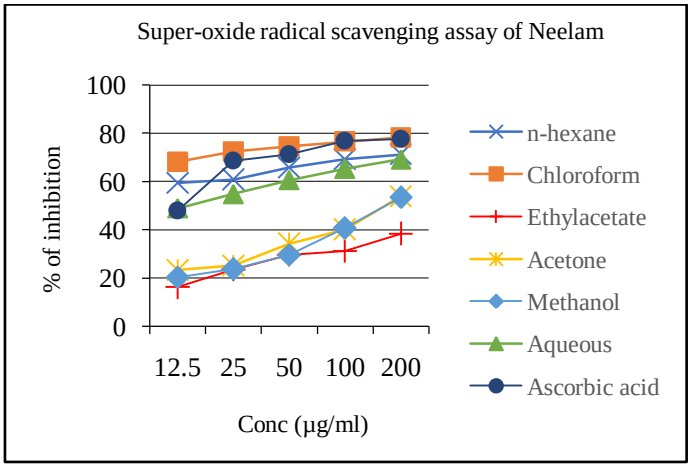

Figure 6 (a)

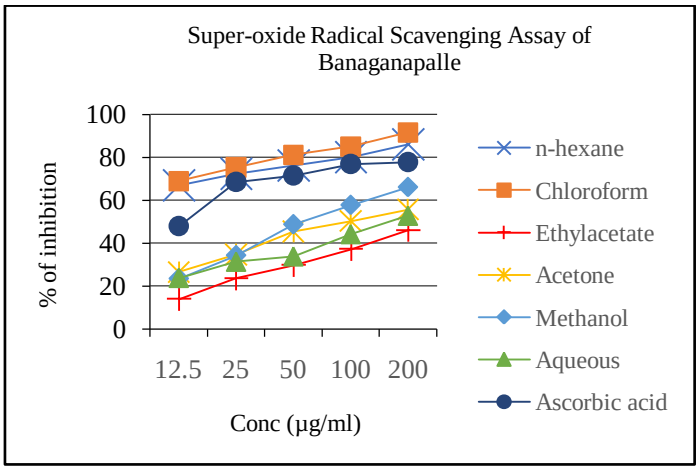

Figure 6 (b)

*III.IV**Superoxide radical scavenging activity:***

Ethyl acetate extract of both the samples showed good superoxide scavenging activities. At 12.5µg/ml concentration, the IC 50 value of Neelam and Banaganapalle were found to be 16.223±0.4856 and 13.9416±0.1341 respectively as compared to the control ascorbic acid whose EC 50 value was 47.9087±0.1756.

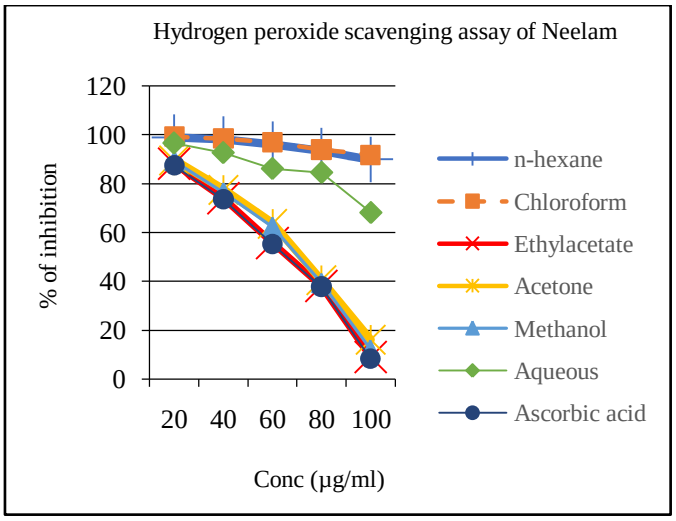

Figure 7 (a)

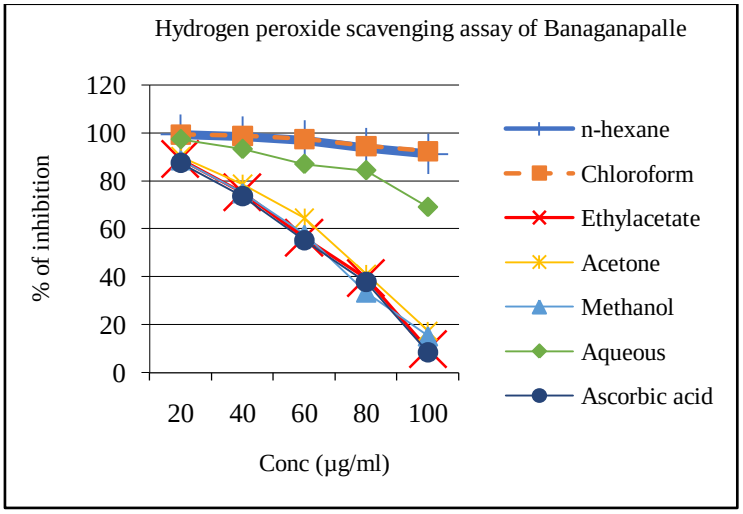

Figure 7 (b)

*III.V**Hydrogen peroxide scavenging activity:***

The MSK extract has shown the hydrogen peroxide scavenging activity in all the sample. The positive control ascorbic acid showed IC 50 value of 8.4027±0.0437 at concentration 100µg/ml whereas the IC 50 value of Neelam and Banaganapalle have found to be 9.0330±0.0509 and 9.7400±0.0252 respectively for ethyl acetate extract.

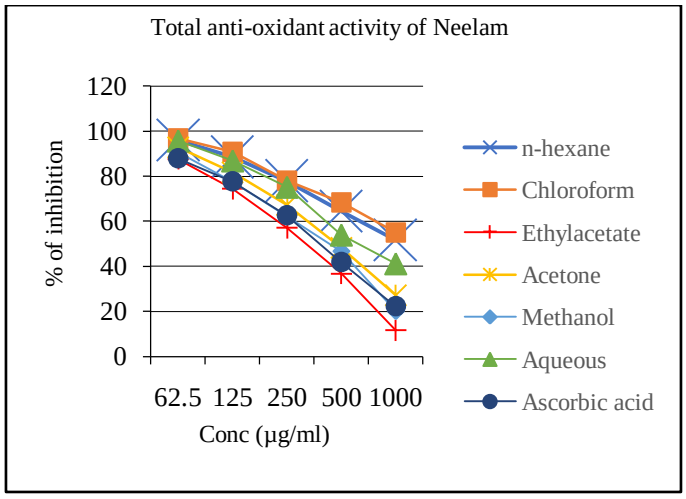

Figure 8 (a)

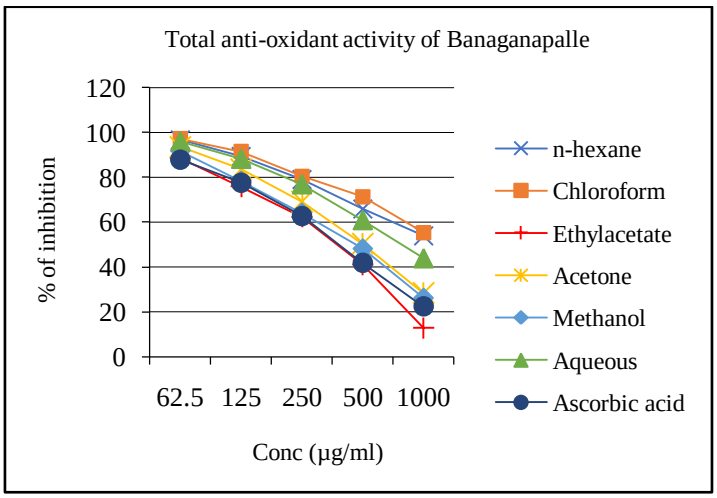

Figure 8 (b)

*III.VI**Total anti-oxidant activity:***

The above two graphs showed the presence of effective anti-oxidant activity in ethyl acetate extract with IC 50 value of 11.702±1.2283 and 12.7659±0.6141 respectively for Neelam and Banaganapalle variety at 1000µg/ml concentration which is lowest among all the value including the control used i.e., Ascorbic acid (IC 50 value 22.3403±1.0638 at 1000µg/ml concentration).

IV. Discussion

Constant production of free radicals is a hallmark of each living system; these radicals can harm tissues and biomolecules, ultimately leading to a wide range of pathologies, notably those characterised by degeneration or widespread lysis [15].

Numerous synthetic medications offer protection against oxidative damage, but they have undesirable side effects. Consuming natural anti-oxidants from dietary supplements and conventional medicine is an alternative remedy to the problem. Consequently, it is evident that MSK have potent anti-oxidant properties.

In the present investigation, it was discovered that mango kernel powder had a strong anti-oxidant capacity. There were minor but substantial distinctions between the two kinds. The methanol, acetone, aqueous, n-hexane, and chloroform extracts had the greatest anti-oxidant activity, followed by the ethyl acetate extracts of both kinds.The antioxidant capacity of mango seed kernel was found to be very high in the present experiment. In a recent study, the ethanol extract (50mg/ml) was shown to have DPPH and reducing assay values of 85.45 and 56.61, respectively [16]. Several other kinds of literature also stated the DPPH and ABTS scavenging ability values of 87.70±.70 and 89.43±.87 respectively in the acetone extract of mango seed kernel [17]. Superoxide radical scavenging assay and hydrogen Peroxide scavenging activity of *Ocimum sanctum* (Tulasi) seed was done and the result showed 31±0.9 and 35±2.1 in (50µg/ml) concentration [18]. Likewise, another report presented the antioxidant activity such as DPPH, ABTS, and total antioxidant activity of values 47.3±0.85, 7.9±0.14, and 4.0±0.11 respectively [19]. The powdered mango kernels were found to have powerful anti-oxidant properties in this study. The two varieties' differences were slight but distinct. The antioxidant activity of the ethyl acetate extracts of both types was inferior to that of the methanol, acetone, aqueous, n-hexane, and chloroform extracts.

V. Conclusion

This antioxidant assay experiment shows that mango seed kernel extract has potent anti-oxidant and free radical scavenging properties. Additionally, it demonstrates reducing power. According to the results of these in vitro anti-oxidant assays, MSK extract is a rich source of natural anti-oxidant that has the potential to halt the development of several oxidative stressors. However, it is not yet known which specific components are responsible for the anti-oxidative effect. Further research is going on in our laboratory to isolate and identify the anti-oxidant chemicals found in the mango seed kernel extracts apart from the above. Further research is going on for therapeutic applications,&in vitro anti-oxidant activity of the extracts.

Acknowledgment

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