COMPARISION OF IN-VITRO ANTIOXIDANT POTENTIAL ACTIVITY OF MICROGREENS

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ABSTRACT:
Microgreens are considered plant sprouts somewhere between sprouts and greens which have an aromatic taste and concentrated food content and come in many colors and textures.
Aim: The aim of this study is to evaluate the in-vitro antioxidant potential activity of selected microgreeens. **Method:** We have choosen soxhlet extraction method for extraction and analysed the the absorbance of microgreens at different concentrations through FRAP (Frap Assay Method)
Result: We were able to obtain mean values of Absorbance of extractions at different concentrations (20,40,80,160,320) µg/ml and the values of Basil are 0.23, 0.27, 0.28, 0.30, 0.32 ; Mustard are 0.12, 0.11, 0.13 ,0.15, 0.12 and Chia are 0.16, 0.14, 0.13 , 0.14 , 0.13. Ascorbic acid which has high anti oxidant potential was taken as standard and based on the given values of absorbance of our microgreens, IC50 was calculated using the standard formula for % inhibition of microgreens and therefore IC50 was found to be at 52,54,54,µg/ml concentrations of mustard ,chia and basil.**Conclusion:** In the present study it is concluded that Mustard microgreen was having high antioxidant potential activity when compared to Chia and Basil microgreens. The microgreens are having moderate antioxidant potential activity when compared to standard ascorbic acid potential activity .

Keywords: Microgreens,antioxidant potential ,inhibitory concentration

INTRODUCTION

(1)Microgreens are young vegetable greens that are approximately 1–3 inches (2.5–7.5 cm) tall. They have an aromatic flavor and concentrated nutrient content and come in a variety of colors and textures. (2)Microgreens are also considered baby plants, falling somewhere between a sprout and baby green. Microgreens are packed with nutrients. (3) Microgreens are also a great source of beneficial plant compounds like antioxidants. Microgreens require slightly less warm and humid conditions than sprouts do, and only the leaf and stem, rather than the root and seed, are consumed. (4) That said, they shouldn't be confused with sprouts, which do not have leaves. Microgreens are usually harvested 7–21 days after germination, once the plant's first true leaves have emerged. Microgreens are more similar to baby greens in that only their stems and leaves are considered edible.(5) However, unlike baby greens, they are much smaller in size and can be sold before being harvested. This means that the plants can be bought whole and cut at home, keeping them alive until they are consumed. (6)Microgreens are very convenient to grow, as they can be grown in a variety of locations, including outdoors, in greenhouses and even on your windowsill. Microgreens can be grown in soil or entirely in medium it doesn't require any chemicals and small amount of water is required for full development of cotyledons with one or two true leaves. It grows within 20- 40 days

2. Selection of Micro green

Mustard Microgreens:

(7)Mustard is an annual, cool-season specialty cash crop that has a short growing season and is commonly grown in rotation with small grains. (8)Mustard is the name given to two closely related species in the Brassica family. (13)Yellow mustard, Sinapis alba L. (also identified as Brassica hirita L.) and Indian,oriental or brown mustard, Brassica juncea L. (9) Mustard Botanical Name- Brown mustard is known by the botanical or scientific name Brassica juncea. Brassica nigra is the scientific name for black mustard. (10)Brassica rapa subsp. oleiferais the scientific name for field mustard. Brassica juncea L. is a member of the Cruciferae or Brassicaceae family of plants and is known by various common names, including Chinese mustard, brown mustard, and oriental mustard. Mustard is native to temperate regions of Europe and has its historic base there.(30)

Chemical Constituents:

(14) Mustard contains numerous phytochemical such as: vitamins, minerals, dietary fiber, chlorophylls, glucosinolates (and their degradation products), polyphenols and volatilecomponents (allyl isothiocyanate, 3-butyl isothiocyanate, etc.The mustard plant is a member of the cruciferous family of vegetables, which also includes broccoli, cabbage, and kale. It is an annual plant that grows to a height of about 2-3 feet and produces small yellow or white flowers.(24) Mustard seeds are used to make mustard, a condiment that is widely popular around the world. Smaller and darker seeds are considered hotter and tastes sharp with a nutty aftertaste. Brown seeds are a little sweeter than the black ones. White mustard seeds are readily available in the market and are sold in whole, crushed or ground form .(25)Major Mustard production states in India:- Rajasthan, Gujarat, Uttar-Pradesh, West Bengal, Haryana, Madhya Pradesh and Assam are major mustard seeds producing states in India. Local Names of Mustard in India:- Rai, Banarasi rai, Kalee sarson (Hindi), Rai (Gujarati),Sasave (Kannada), Assue,Sorisa (Kashmiri), Avalu (Telugu), Kadugo (Tamil), Kaduku(Malayalam), Rai, Banarasi rai, Kalee sarson (Punjabi)

Chia Microgreens:

(12)Chia is a warm-climate annual herb that is indigenous to Mexico and Guatemala. The name is derived from a Mayan word that means “strength” and an Aztec word that means “oily”. Its botanical name, Salvia hispanica, also refers to it. (23)The largest genus in the lamiecece,or mint, family is salvia. As with many mint plants, these types are known for their assertive growth and, in some cases, aggressive spread. (15)Once established, chia plants require hardly any care. Flowers form on spikes resembling wheat on stalks that can reach a height of five feet. Chia plant maintenance is simple. Not only is the desert plant drought-tolerant, but it is also renowned as a “fire following” plant, which means it is one of the first to return after a disastrous wildfire. After the plants have been established in well-drained soil, water them infrequently. (22)Chia plants are so versatile that they can self-pollinate in the absence of bees or butterflies, and they will self-sow the next autumn if they survive the predation of birds, insects, and animals.

Chemical Constituent:

(19) Gallic, caffeic, chlorogenic, cinnamic and ferulic acids, quercetin, kaempferol, epicatechin, rutin, apigenin and p-coumaric acid It grows in clay or sandy well-drained soils, in sunny places, between 1000 and 2750 meters. It requires a tropical or subtropical climate. (18)It does not tolerate frost. It is a plant native to tropical America, a a part of the diet of the natives, together with quinoa and amaranth cereals that, like chia, were banned by the settlers because they were part of the pagan rituals.(16) Nowadays, chia grows as a wild plant in l tropical forests, thorn forests and also, cloud forests, forests of oak, pine and juniper forests **Basil Microgreens**:(17)Basil is also Ocimum basilium also called great basil, is aculinary herb of the family Lamiaceae (mints). It is a tender plant, and is used in cuisines worldwide. In Western cuisine(21)the generic term "basil" refers to the variety also known as sweet basil or Genovese basil. Basil is native to tropical regions from Central Africa to Southeast Asia. In temperate climates basil is treated as an annual plant, however, basil can be grown as short lived perennial or biennialin warmer horticultural zone with tropical or Mediterranean climates. Basil is an annual, or sometimes perennial, herb used for its leaves. (29) Depending on the variety,plants can reach heights of between 30 and 150 cm (1 and 5

ft). Its leaves are richly green andovate, but otherwise come in a wide variety of sizes and shapes depending on cultivar. Leaf sizes range from 3-11 cm (1 to 4+½ in) long, and between 1 and 6 cm (½ and 2+½in) wide. Basil grows a thick, central taproot. Its flowers are small and white, and grow from a central in florescence ,or spike, that emerges from the central stem atop the plant. Unusual among Lamiaceae , the four stamens and the pistil are not pushed under the upper lip of thecorolla,but lie over the inferiorChemical Constituents:
(20)The major constituents were found to be methyl cinnamate (70.1%), linalool (17.5%), β-elemene (2.6%) and camphor (1.52%). (26) Garden plant, native from India. Annual herb up to 1 m. Erect stems, round below, squared above. Ovate or lanceolate leaves, opposite till 5 cm long, long petiolated , darker above. Botanical description of Basil Very odorous flowers grouped in spikes, loose verticils with 6 flowers each. Calyx with 6 lobes, edge ciliated. White or pink corolla, stamens white. Upper lip with 4 lobes, lower one undivided. Picking-up and storing basil leaves and flowers. Leaves and floral summits should be collected in summer. They must be dried in the shade and be kept in crystal containers very well closed. Basil is native to tropical regions from Central Africa to Southeast Asia. In temperate climates basil is treated as an annual plant, however, basil can be grown as a short-lived perennial or biennial in warmer horticultural zones with tropical or Mediterranean climates . (27)Basil is known as the “King of Herbs” in part due to its popularity but also as a result of its name basilicum .basil loves warm weather, a hot midday sun can be rather harsh. If you see wilting only during the peak hours for summer temperatures, you may need to add light shade over your basil plant. Active components of Basil Essence with linaloal, estragole and eugenol.Calyx with 6 lobes, edge-ciliated. White or pink corolla, stamens white.

METHODOLOGY

(11)Leaves of these microgreens were collected from our college garden at MRIPS maisammaguda, secunderabad. Leaves of Mustard microgreen, Chia microgreen, Basil microgreen were collected cleaned, dried under shade for around 3days.
Dried leaves were grounded into coarse powder by using grinder.

Mustard Microgreens:

Before drying- 58.70gm
After drying- 10.70gm

Loss on drying= Initial weight –final weight / Initial weight x 100

Loss on drying = 40.47 %

Basil Microgreens :

Before drying- 61.28
After drying- 11.60gm
Loss on drying = 42.35%

Chia Microgreens :

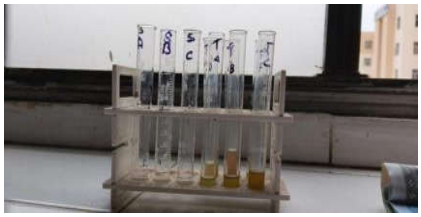
Before drying- 50.28
After drying- 8.60gm
Loss on drying = 33.17 %

The powder was subjected for extraction by Soxhlet apparatus using methanol as solvent for About 30minutes.

Chemicals And Reagents:80% MethanolPBS (Phosphate buffer solution)**,**Potassium ferricyanide**,**TCA (Tricyclic acetic acid)**,**Ferric chloride **.**Distilled water

The antioxidant activity of microgreen samples were evaluated using FRAP (Ferric Reducing antioxidant power) (28) assay according to standard literature. Micro green extracts are taken in various concentrations(20, 40, 80,160, 320) Volume of each sample with different concentrations is made to 1 ml using the same solventemployed for extraction of sample. same solvent employed for extraction of sample.Thoroughly mix the contents in each tube.Then, 2.5 ml of 1% potassium ferri cyanide solution is added in all different concentrations amples and each reaction mixture is vortexed well using a vortex shaker.The samples are incubated at 50 °c for around 20 minutes.Once the incubation time is over, 2.5 ml of 10% TCA is added in each sample.The test tubes are centrifuged at 3,000rpm for 10 minutes.From these centrifuged samples, 2.5 ml from supernatant is collected in separate tubes.Then, to these new separate test tubes, we add 5 ml of ferric chloride.This would give us bluish colour formation.And then take the measurement at 700nm

RESULTS AND DISCUSSION



preparation of concentrations of chia, basil and mustard microgreens extracts

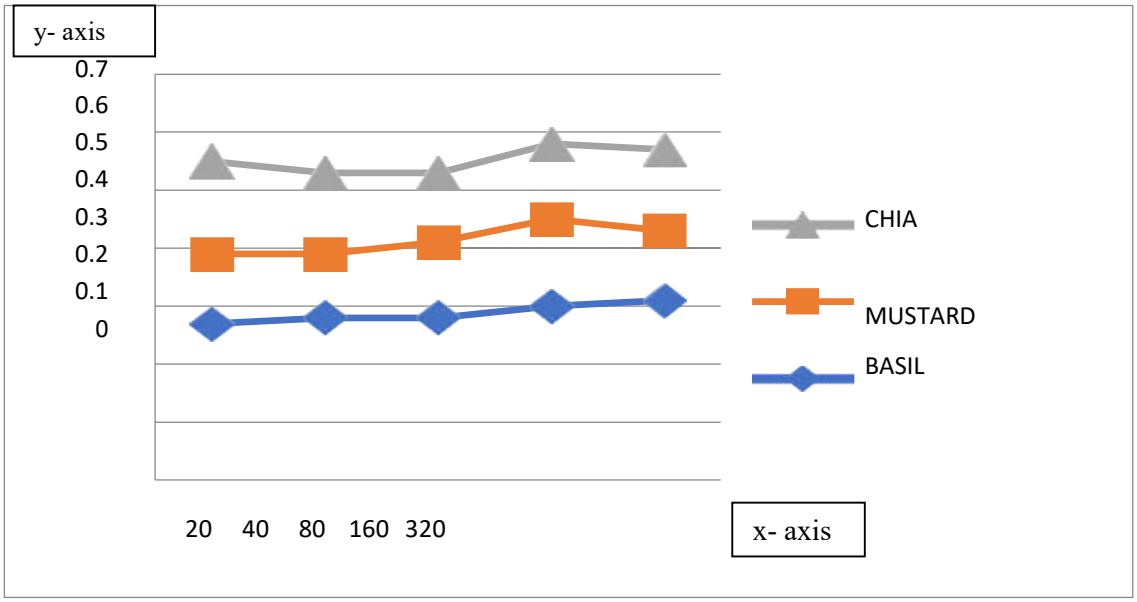
TABLE -1

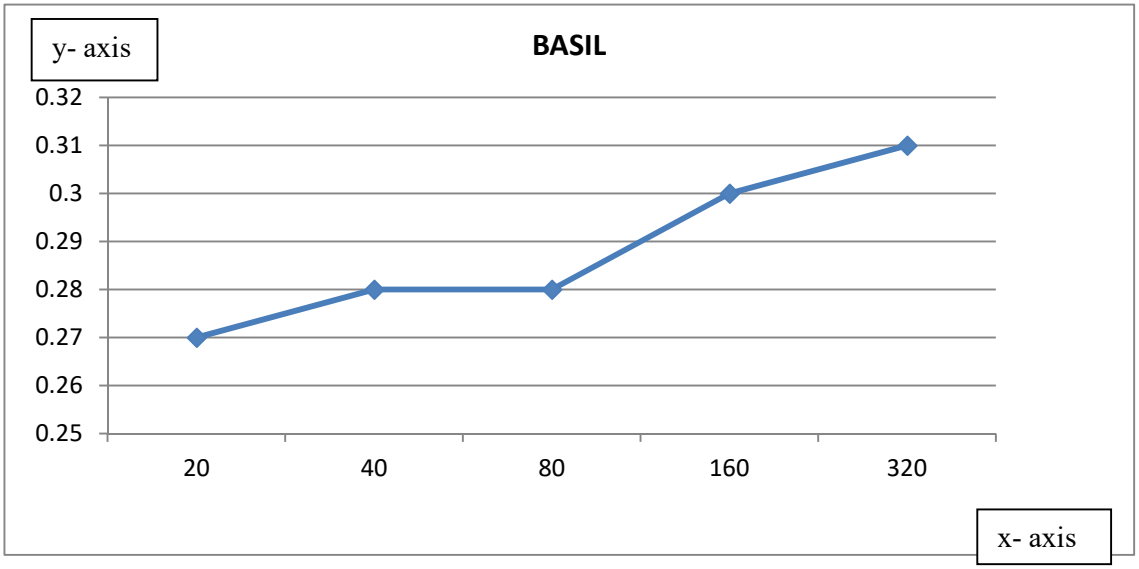
MICROGREENS	DIFFERENT CONCENTRATIONS OF EXTRACTS AT µg /ml				
	20	**40**	**80**	**160**	**320**
Basil Absorbance	0.28	0.28	0.28	0.30	0.31
	0.26	0.29	0.27	0.31	0.32
	0.27	0.27	0.29	0.32	0.30
Mustard absorbance	0.12	0.11	0.13	0.15	0.13
	0.11	0.10	0.12	0.14	0.12
	0.13	0.12	0.14	0.16	0.11
Chia absorbance	0.15	0.14	0.13	0.14	0.14
	0.17	0.13	0.12	0.13	0.13
	0.16	0.15	0.11	0.12	0.15

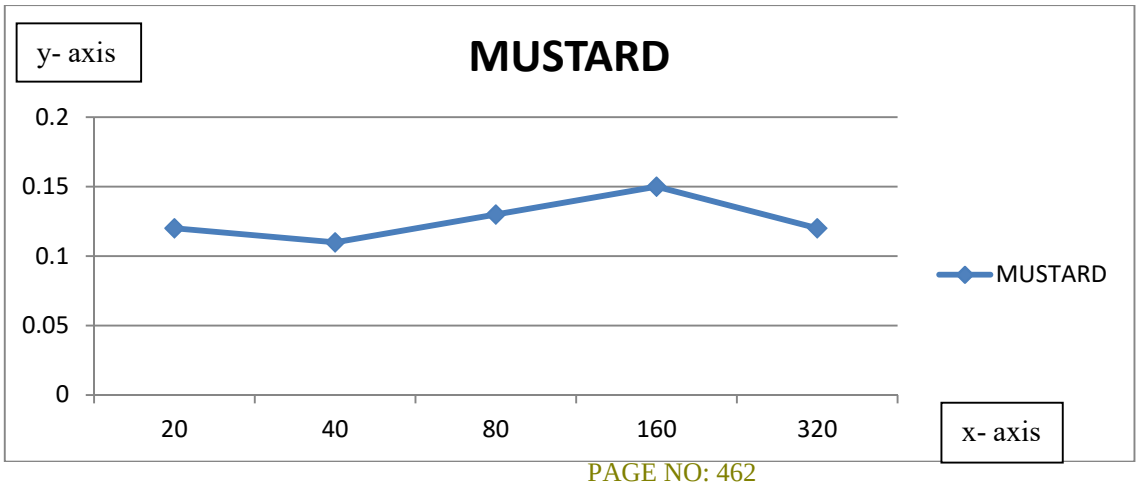
TABLE - 2

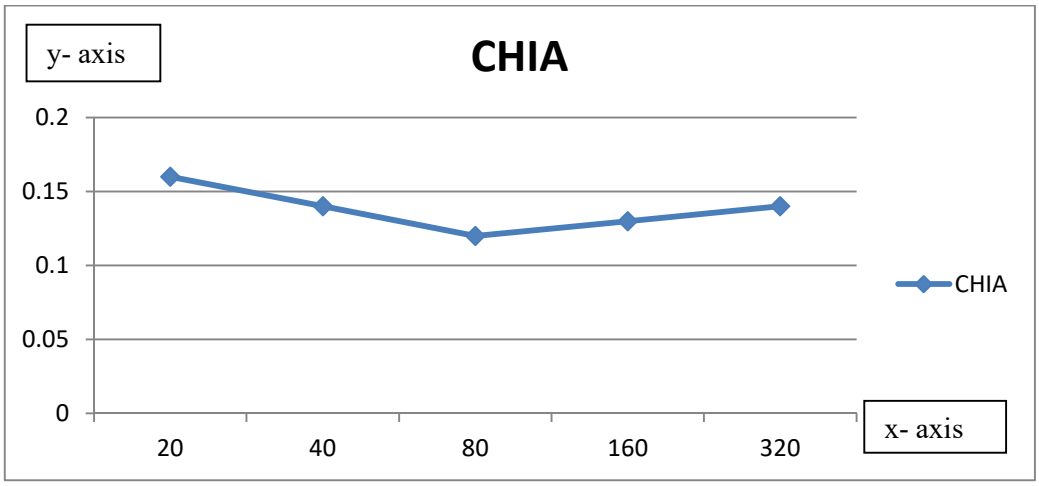
Microgreens	**MEAN VALUES AT DIFFERENT CONCENTRATIONS OF EXTRACTS AT µg/ml**				
	20	40	80	160	320
Basil Absorbance	0.27	0.28	0.28	0.30	0.31
Mustard Absorbance	0.12	0.11	0.13	0.15	0.12

Chia Absorbance	0.16	0.14	0.12	0.13	0.14





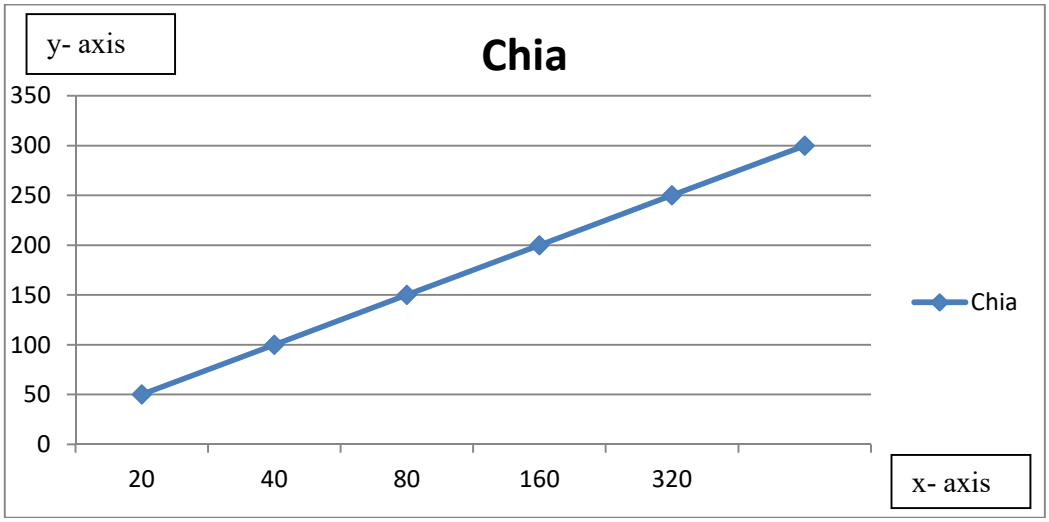


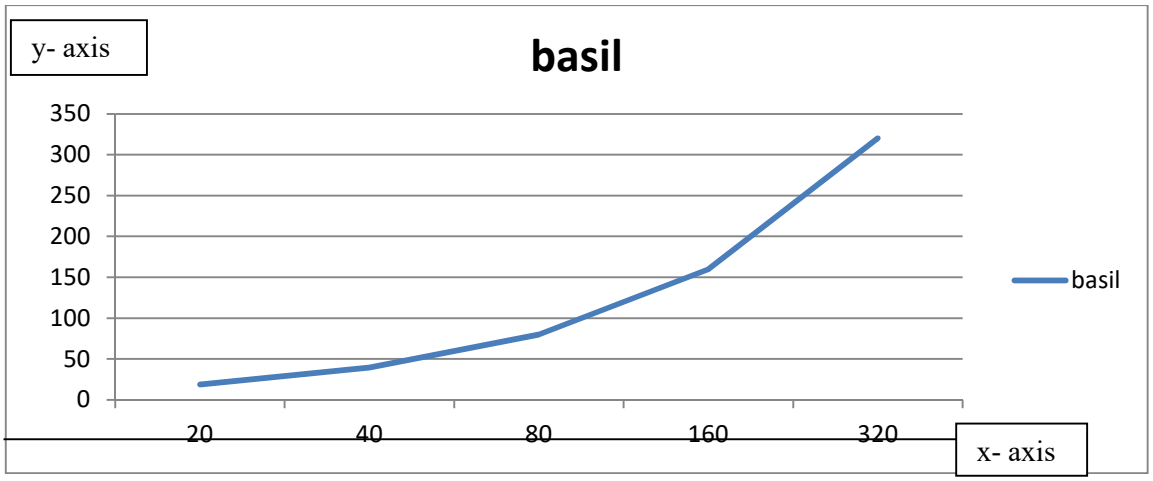


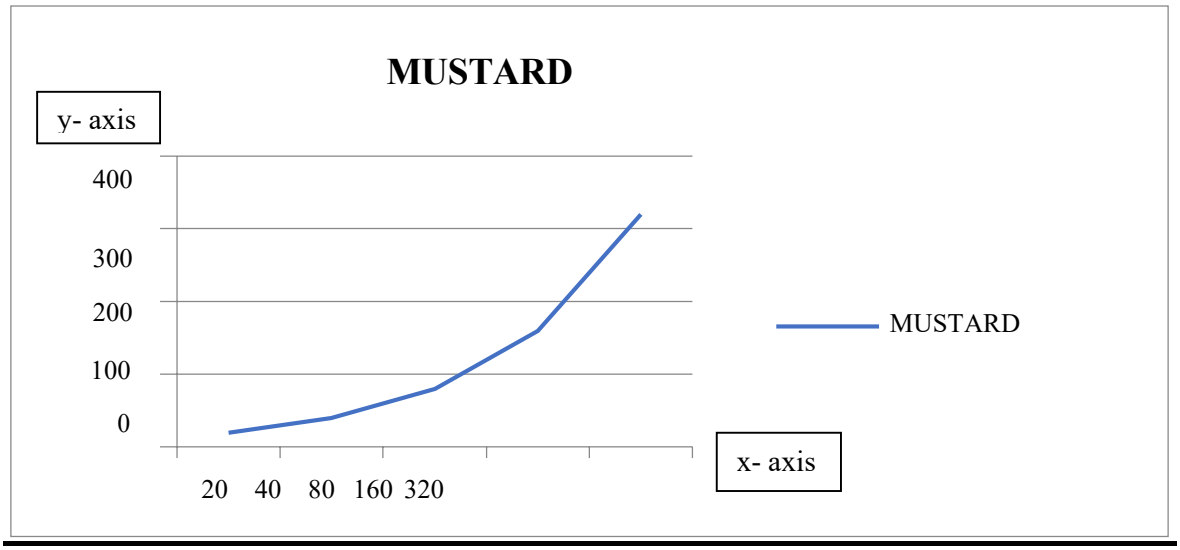
Formula for %inhibition:

$$\%\text{Inhibition}=\{(Ao - As)/Ao\}\ X\ 100$$

Concentration of drug	Percentage inhibition		
	Basil	mustard	chia
20	18.6	19.4	19.2
40	39.3	39.7	39.6
80	79.65	79.85	79.8
160	159.85	159.92	159.9


y- axis
Chia
350
300
250
200
150
100
50
0
20
40
80
160
320
Chia
x- axis





According to the standard literature the study of microgreens have indicated the potential anti oxidant, anti cancer, anti inflammatory, antiseptic, aphrodisiac, astringent, bitter, demulcent, emollient effect. The results of Comparative Analysis of Antioxidant properties of Chia and Basil study correlates the total antioxidant capacity (TAC) of tea and basil seeds with the help of various tests. In a previous study, we found that the TAC (Total Antioxidant Capacity) was seen when Chia and Basil seeds were mixed in fixed proportions ,and when the seeds were soaked. The review of phytochemistry and biological activity of Mustard seeds provides an overview of mustard's botanical characteristics, species, origin, distribution and uses, as well as its phytochemicals and biological activities. T he results of this study can provide information for the development and use of mustard sourc es. Lipid peroxidation is the chain of reactions of oxidative degradation of lipids. It is the process in which free radicals "steal" electrons from the lipids in cell membranes resulting in cell damage antioxidants present in microgreens act by inhibiting the propagation step of lipid peroxidation hence preventing cell damage.We have focused on the potential antioxidant activity of the microgreens using soxhlet extraction method. Soxhlet extraction using 80% methanol have been choosed to obtain the extract. We have used FRAP assay method to determine the absorbance of microgreens at different concentrations. We were able to obtain mean values of Absorbance of extractions at different concentrations (20,40,80,160,320) µg/ml and the values of Basil are 0.23, 0.27, 0.28, 0.30, 0.32 ; Mustard are 0.12, 0.11, 0.13 ,0.15, 0.12 and Chia are 0.16, 0.14, 0.13 , 0.14 , 0.13. Ascorbic acid which has high anti oxidant potential was taken as standard and based on the given values of absorbance of our microgreens, IC50 was calculated using the standard formula for % inhibition of microgreens and therefore IC50 was found to be at 52,54,54,µg/ml concentrations of mustard ,chia and basil.

CONCLUSION

Mustard, chia, basil {microgreens} are used as herbal medicine due its wide range of phytoconstituents and pharmacological properties. Therefore a few reports on phytochemical analysis of seed's extract with aqueous methanol (80%solvent) of microgreens have been observed. We investigated and collected the whole microgreens from college garden of MRIPS campus. This method has been done by using aqueous methanol (80%solvent) and was evaluated for antioxidant potential activity by soxhlet extraction and absorbance by FRAP assay method was done with the help of standard literature. The extract consist of antioxidant and several other phytoconstituents, We promote the usage of the microgreen seeds of Mustard, Chia, and Basil seeds in daily intake to promote the antioxidant potential which will have a high impact on ageing process, cancer deformation and effects on autoimmune disorders. So the present study provides evidence that aqueous methanolic extract(80%) of microgreens {mustard, chia, basil } contains medicinally important antioxidant compounds and Mustard microgreen was having high antioxidant potential activity when compared to Chia and Basil microgreens. The microgreens are having moderate antioxidant potential activity when compared to standard ascorbic acid potential activity . Lot more studies has to be done to develop the formulation of the microgreen extracts of Mustard, Chia and Basil to promote health care in a safer and effective way.

FIGURES

Figure 1 – Microgreens

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