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An Evaluation of Hibiscus Sabdariffa Flowers as Antioxidant Agent on Microwave Treated Polymethyl Methacrylate Powder In vitro

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Abstract: Hibiscus Sabdariffa flowers was used in traditional medicine. This study would contribute new additional knowledge on the bioactivity antioxidante property of Hibiscus Sabdariffa alone and with acrylic denture base after exposure of PMMA to the microwave energy and reducing particle size with grinding by adding natural additives (Hibiscus Sabdariffa and Vanillin) with different concentrations.

Materials and Methods: PMMA powder was treated with microwave radiation at a power level of 360watt for ½ hr. The obtained PMMA powder and the additives (Hibiscus Sabdariffa and Vanillin) were then grinded using an electrical blender individually. The next step is particle size reduction of the microwave treated PMMA powder and additives to 80µm individually also. Then mixing the control PMMA powder and the treated PMMA powder with additives in different concentrations, and making acrylic samples with different concentrations of additives and divide the work into groups of acrylic samples and pure materials which include (acrylic powder, acrylic microwave powder, cured acrylic, cured acrylic microwave, pure H 0.05%, pure H 0.01%, pure V 5%, pure V 0.1%, acrylic microwave +H 0.05%, acrylic microwave+H 0.01%, acrylic microwave +H 0.05% +V 0.1% and acrylic microwave +H 0.05% +V 5%) and tested by Ferric reducing power (FRP) assay to evaluate the antioxidant activity.

Results: according to the statistical analysis (One way ANOVA and Duncan's Multiple Range Test), there is highly significant difference between the groups and show high antioxidant activity with pure H 0.05% and low activity with pure V 0.1% and after adding the additive on the acrylic with microwave also have high antioxidant activity.

Conclusion: The use of Hibiscus Sabdariffa flowers useful in obtaining antioxidant activity as pure natural material and with acrylic resin which considered cheap natural pigment and more economic and may be helpful in patients with oral cancer and enhance the oral tissue.

Keywords: Antioxidant Activity, FRP Assay, Hibiscus Sabdariffa, Microwave, PMMA Powder, Vanilla.

INTRODUCTION

Polymethyl methacrylate (PMMA) resins have dominated the denture base market for more than 50 years. This was due to PMMA's good physical properties, availability, reasonable cost and ease of manipulation [1]. The use of microwave radiation produce an effect on the acrylic powder, and this effect improve the transverse strength and residual monomer concentration of the denture base acrylic specially for 360 watt power that treated with microwave for ½ hr. [2]. Microwave heating is independent of thermal conductivity. The advantages of curing denture base resin by microwave energy include greatly induced curing time, less cumbersome equipment, and a cleaner method of processing. Other advantages claimed, but not substantiated, include a shortened dough forming time, more homogeneous resin dough, and minimal color change in resin base [3].

An antioxidant is a chemical that prevents the oxidation of other chemicals. They protect the key cell components by neutralizing the damaging effects of free radicals, which are natural by- products of cell Metabolism [4].

Oxidative stress is caused by an insufficient capacity of biological systems to neutralize excessive free radical production, which can contribute to human diseases and aging [5]. Nutritional antioxidant deficiency also leads to

oxidative stress, which signifies the identification of natural anti-oxidative agents related to phenolic compounds present in die consumed by human population [6, 7].

Phenolic compounds are known to counteract oxidative stress in the human body by helping maintaining a balance between oxidant and antioxidant substances [8, 9].

Hibiscus Sabdariffa named roselle, is an annual herbaceous shrub, cultivated for its flowers although leaves and seeds have also been used in traditional medicine [10].There are many species of *Hibiscus sabdariffa* use as strong antioxidant and antitumor [11].

The antioxidant activity of roselle extract is also pH dependent (pH 2 to 7), the activity decreases as pH increases. However, at a constant pH, only a relatively small decrease in antioxidant activity and total phenolic content is observed [12].

Vanilla is a crop of great commercial importance as the source of natural vanillin, a major component of flavor industry [13].

There are many compounds present in the extracts of vanilla, the compound is vanillin (4-hydroxy-3-methoxybenzaldehyde) is the major component of natural vanilla [14],

It is also used in the fragrance industry, in perfumes, livestock fodder, and cleaning products. However, Vanillin has been used as flavor resources in confectionery, food, sensual desserts such as ice cream, sugar cookies, puff pastries, and butter creams and in pharmaceutical preparations [15].

Ferric reducing power assay is a colorimetric assay that measures the ability of plasma to reduce the intense blue ferric tripyridyltriazine complex to its ferrous form, thereby changing its absorbance [16] and measures total reductive power [17] in plasma, but the assay subsequently has also been adapted and used for the assay of antioxidants in botanicals [18].

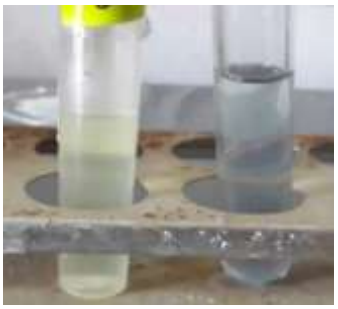
So the purpose of this study to evaluate in vitro test of biological property of antioxidant activity of *Hibiscus Sabdariffa* alone and with acrylic denture base after exposure of PMMA to the microwave energy and reducing particle size with grinding by adding natural additives (*Hibiscus Sabdariffa* and Vanillin) with different concentrations.

MATERIALS AND METHODS

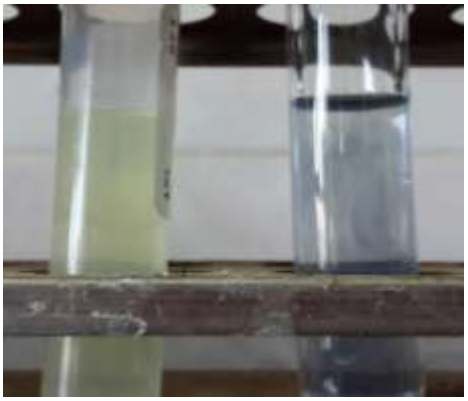
A 50 gm of PMMA powder (Vertex™-Dental by Johan) is used as constant weight for preparation of each group of the tested material. PMMA is prepared in wet condition and put in microwave at 360 watt 40 % level of power then removed from microwave and after material exposure to microwave radiation, it removed and crushed immediately by electrical grinder (Clatronic, germany) for 5 minutes or until the most solid pieces are grinding [2], then sieved by the size of 100 µm sieve after that sieved by smaller size sieve (80µm) after grinding of treated PMMA [19] *Hibiscus sabdariffa* (from Sudan) is used as dry flowers, these flowers grind in electrical grinder and then sieved by the size of 80 micron sieve. Vanillin also used as crystals powder, this powder grind in electrical grinder for 5± 0.5 min. then sieved by the size of 80 micron sieve. This procedure occurred in Alhokamaa Company for drug industry and medical supplies in Ninava. Then mixing acrylic monomer with hibiscus that give the red color in tow concentrations (0.01%, 0.05%) alone [19]. Another additives vanillin used with concentration (0.1%, 5%) [20] with additive of *Hibiscus sabdariffa* (0.05%) in clean dry gar and prepared the samples of experimental groups and the pure materials for FRP assay.

Ferric reducing power (FRP) was assessed using the potassium ferrocyanide assay [21]. Different dilutions of extract (10 ml, 10%) of Hibiscus sabdariffa flower powder with different concentrations (0.05, 0.01 %) and from vanillin powder (5%, 0.1 %), and also prepared extracts from all groups of the study and control groups were added to 2.5 ml phosphate buffer (0.2 M and pH 6.6) and 2.5 ml of potassium ferricyanide (1% w/v). The mixture was incubated at 50°C for 20 min. Trichloroacetic acid solution (2.5 ml and 10% w/v) was added to stop the reaction. The mixture was then separated into aliquots of 2.5 ml and diluted with 2.5 ml of water. To each diluted aliquot, 0.5 ml of ferric chloride solution (0.1% w/v) was added. After 30 min, absorbance was measured at 700 nm [22] and a higher absorbance indicates a greater reducing power [23] (figure 1), this test was done by using ph meter to regulate phosphate buffer, and spectrophotometer devices.

A: sample of PMMA powder



B: sample of PMMA after curing



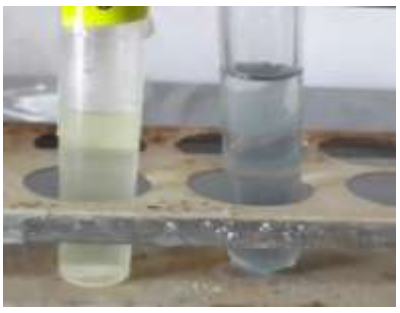
C: sample of PMMA powder after exposure to microwave radiation

E: sample of cured PMMA after exposure to microwave radiation

F: sample of Hibiscus Sabdariffa 0.05%

G: sample of Vanilla 0.1%

H: sample of cured microwavable PMMA + H 0.05%



I: sample of cured microwavable PMMA + H 0.05% + V 0.1%

Figure 1: Ferric reducing power (FRP) of prepared samples (A-I)

RESULT

Ferric reducing power assay of twelve groups in this study. Mean stander deviation and Duncan test of acrylic resin. These statistical analysis of antioxidant activity were shown in figure (2).

One way ANOVA analysis of variance test show that there is a highly significant differences at P<0.05 (acrylic powder, acrylic micro powder, cured acrylic, cured acrylic micro, Hibiscus Sabdariffa 0.05%, Hibiscus Sabdariffa 0.01%, Vanilla 5% and Vanilla 0.1%) and part two include (Hibiscus Sabdariffa 0.05%, Hibiscus Sabdariffa 0.01%, Vanilla 5%, Vanilla 0.1%, acrylic micro+ Hibiscus Sabdariffa 0.05%, acrylic micro+ Hibiscus Sabdariffa 0.01%, acrylic micro+ Hibiscus Sabdariffa 0.05%+ Vanilla 0.1% and acrylic micro+ Hibiscus Sabdariffa 0.05%+ Vanilla 5%). From Duncan's Multiple Range Test in part one (acrylic powder, acrylic micro powder, cured acrylic, cured acrylic micro, Hibiscus Sabdariffa 0.05%, Hibiscus Sabdariffa 0.01%, Vanilla 5% and Vanilla 0.1%) showed significant increase of Hibiscus Sabdariffa 0.05% as pure material and significant decrease of Vanilla 0.1% as pure material also and the other groups graduated from two these values as shown in the table (1).

And in part two (Hibiscus Sabdariffa 0.05%, Hibiscus Sabdariffa 0.01%, Vanilla 5%, Vanilla 0.1%, acrylic micro+ Hibiscus Sabdariffa 0.05%, acrylic micro+ Hibiscus Sabdariffa 0.01%, acrylic micro+ Hibiscus Sabdariffa 0.05%+ Vanilla 0.1% and acrylic micro+ Hibiscus Sabdariffa 0.05%+ Vanilla 5%) showed significant increase of Hibiscus Sabdariffa 0.05% as pure material and significant decrease of Vanilla 0.1% as pure material and after adding these materials to the acrylic showed significant increase in the antioxidant value in comparing to the acrylic without these additives as shown in the table (2).

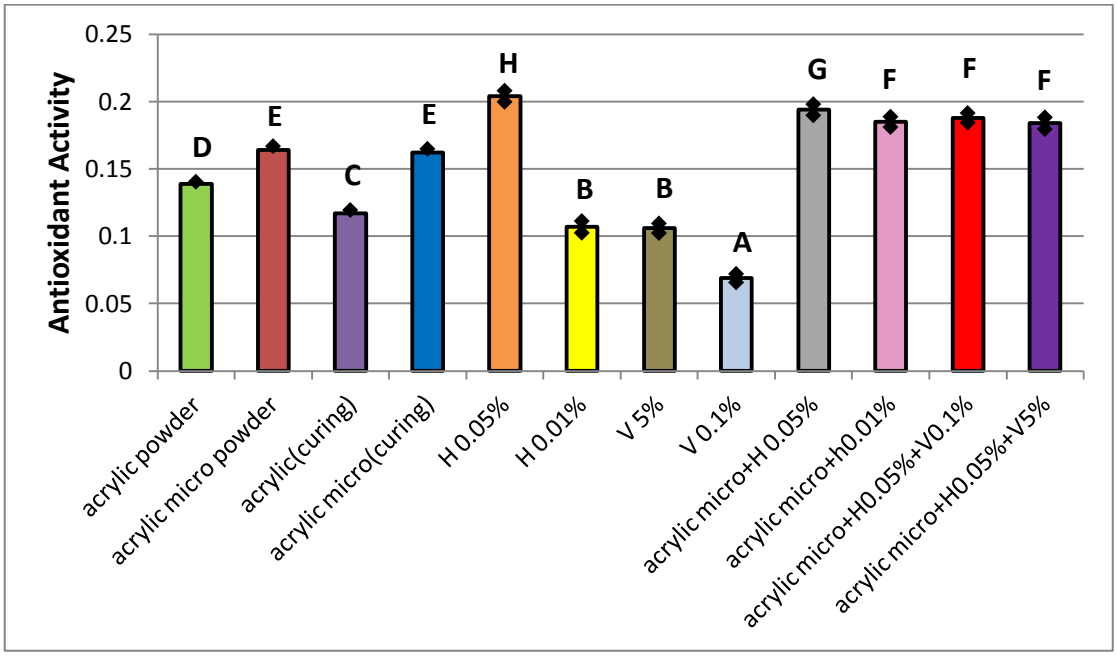


Figure 2: Antioxidant activity of mean, standard deviation and Duncan's multiple range test.
***different letters means significant differences between tested groups at p≤ 0.05. H: Hibiscus Sabdariffa, V: Vanilla.**

Table 1: One way ANOVA of antioxidant activity test of (acrylic powder, acrylic micro powder, cured acrylic, cured acrylic micro, Hibiscus Sabdariffa 0.05%, Hibiscus Sabdariffa 0.01%, Vanilla 5% and Vanilla 0.1%).

SOV	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.063	7	.009	854.370	.000
Within Groups	.000	32	.000		
Total	.064	39			

* Significant difference at p≤ 0.05. df: Degree of freedom, SOV: Source of Variance.

Table 2: One way ANOVA of antioxidant activity of (Hibiscus Sabdariffa 0.05%, Hibiscus Sabdariffa 0.01%, Vanilla 5%, Vanilla 0.1%, acrylic micro+ Hibiscus Sabdariffa 0.05%, acrylic micro+ Hibiscus Sabdariffa 0.01%, acrylic micro+ Hibiscus Sabdariffa 0.05%+ Vanilla 0.1% and acrylic micro+ Hibiscus Sabdariffa 0.05%+ Vanilla 5%).

SOV	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.094	7	.013	886.626	.000
Within Groups	.000	32	.000		
Total	.095	39			

* Significant difference at p≤ 0.05. df: Degree of freedom, SOV: Source of Variance.

DISCUSSION

Antioxidant is a molecule that inhibits the oxidation reaction which produce the free radicals and cause damage to the cell, these agents remove these radicals and called reducing agents likes polyphenolic acid, flavonoids and anthocyanins that may act as antioxidants or have other mechanisms contributing to the cardio protective actions [24] as talking about Hibiscus Sabdariffa in this study as antioxidant agent.

According to this study as shown in the figure (1) of ferric reducing power assay show a highly significant increase of reducing capacity of Hibiscus Sabdariffa powder 0.05% of ethanol extract and significantly decrease when reduce the concentration of Hibiscus Sabdariffa powder to 0.01% of ethanol extract but when adding this Hibiscus powder in two concentration (0.05%, 0.01%) to acrylic with microwave show significant increase in reducing capacity of the new acrylic and the reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity [25]. This antioxidant activity of Hibiscus Sabdariffa due to the phenolic compounds that isolated from *Hibiscus* which include quercetin, luteolin glucoside, and chlorogenic acid in addition to other recorded compounds like the anthocyanins delphidin-3-sambubioside, cyanidin3-sambubioside, flavonoids, gossypetine, hibiscetine and sadderetine [26]. Anthocyanins and other phenolic compounds are the major source of antioxidant capacity in Roselle extract [27]. These phenolic compounds demonstrated protective effect against tert-butyl hydroperoxide (t-BHP) induced oxidative damage and hepatotoxicity both in vitro and in vivo [28].

Also Vanilla powder at concentration (5%, 0.1%) of ethanol extract show significantly decrease gradually in antioxidant capacity compared to the Hibiscus Sabdariffa flower which contained highly amount of phenolic compounds but when adding Vanilla powder in two concentrations (5%, 0.1%) respectively to the acrylic with microwave in addition to Hibiscus Sabdariffa 0.05%, there is high significant increase in antioxidant capacity but also the Vanilla powder still show antioxidant activity but less than Hibiscus Sabdariffa according to this study, this reduction may be related to alcoholic extract show less activity than natural vanillin and the activity increased with increasing concentration of vanilla [29].

In this study also we research in new field of the antioxidant activity of the acrylic itself as a powder (PMMA), after manipulating by microwave energy and also after curing and polymerization, these results include the acrylic powder (PMMA) after exposure to microwave energy show significantly increase in antioxidant activity compared with acrylic alone (PMMA) and after the curing and polymerization of both groups show slight decrease in antioxidant activity but still have reducing property.

Antioxidant according to powder and acrylic treated with microwave, this treatment lead to decrease particle size from 100 to 80 µm and this could improve its properties.

CONCLUSION

The use of Hibiscus Sabdariffa flowers useful in obtaining antioxidant activity as pure natural material and with acrylic resin which considered cheap natural pigment and more economic and may be helpful in patients with oral cancer and enhance the oral tissue.

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REFERENCES

[1]. Gladstone S, Sudeep S, Kumar GA, "An evaluation of the hardness of flexible Denture Base Resins". Health Sciences. 2012;1(3):1-8.
[2]. Ebraheem SN, Hatim NA, Taqa AA, "An evaluation of microwave radiation effect on dry and wetpolymethyl methacrylate powder". 2014;
[3]. Hasan HR, "Comparison of some physical properties of acrylic denture base material cured by water bath and microwave techniques".Al–Rafidain Dent. 2003;3(2):143-147.
[4]. Shenoy R, Shirwaikar A, "Anti-inflammatory and free radical scavenging studies of Hyptis suaveolens (labiatae)". Indian drugs. 2002;39:574 – 577.
[5]. Flora SJ, "Role of free radicals and antioxidants in health and disease". Cell. Mol. Biol. 2007;15,53(1):1–2.
[6]. Manach C, Scalbert A, Morand C, Remesy C, Jimenez L, "Polyphenols: food sources and bioavailability". Am. J. Clin. Nutr. 2004;79(5): 727-777.
[7]. Badarinath AV, Mallikarjuna KRAo, Madhu Sudhana Chetty C, Ramkanth S, Rajan TVS, Gnanaprakash K, "A Review on In-vitro Antioxidant Methods: Comparisions, Correlations and Considerations". Int.J. PharmTech Res. 2010;2(2):1276-1285.
[8]. Materska M, Perucka I, "Antioxidant activity of the main phenolic compounds isolated from hot pepper fruit (Capsicum annuum L.)". J. Agric. Food Chem. 2005;53(5):1750-1756.
[9]. Siddhuraju P, " The antioxidant activity and free radical-scavenging capacity of phenolics of raw and dry heated moth bean (Vigna aconitifolia) (Jacq.) Marechal seed extracts". Food Chem. 2006;99(1):149-157.
[10]. Mahadevan N, Shivali, and Kamboj P, "Hibiscus sabdariffa Linn.- an overview". IJNPR. 2009;8(1):77.
[11]. Özmen A, "Cytotoxicity of Hibiscus rosa-sinensis flower extract". CARYOLOGIA. 2010;63(2):157-161.
[12]. Sukhapat N, Ungphaiboon S, Itharat A, Puripattanavong J, Pinsuwan S, "Influence of pH on antioxidant activity of roselle (Hibiscus sabdariffa L.) extract in aqueous solution". The 10th World Congress on Clinical Nutrition: Nutrition in the Next Decade: Nutraceutical/Functional Food: Product Performance in Health, Disease and Safety. Abstract book. Organized by PSU, INC and BIOTEC, 30 Nov-3 Dec., 2004. Phuket, Thailand. p.184.
[13]. Walton NJ, Mayer MJ, and Narbad A, "Vanillin". Phytochemistry. 2003; 63(5):505-515.
[14]. Podstolski A, Havkin-Frenkel D, Malinowski J, Blount JW, Kourteva G, Dixon RA, "Unusual 4-hydroxybenzaldehyde synthase activity from tissue cultures of the vanilla orchid Vanilla planifolia". Phytochemistry. 2002;61(6):611-620.
[15]. Jadhav D, Rekha BN, Gogate PR, Rathod VK, "Extraction of vanillin from vanilla pods: A comparison study of conventional soxhlet and ultrasound assisted extraction". J Food Eng. 2009;93(4):421-426.
[16]. Benzie IF, Strain JJ, "(1999): Ferric reducing/antioxidant power assay; direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration". Methods enzymol. 1999;299:15–27.
[17]. Pérez-Jiménez J, Saura-Calixto F, "Effect of solvent and certain food constituents on different antioxidant capacity assays". Food Res. Int. 2006;39(7):791–800.
[18]. Benzie IFF, Szeto YT, "Total antioxidant capacity of teas by the ferric reducing/antioxidant power assay". J. Agric. Food Chem. 1999;47(2):633-636.
[19]. Qustanteen FR, Hatim NA, "An evaluation of color property of hibiscus sabdariffa flowers on microwave treated polymethyl methacrylate in vitro and vivo by 3d computer program". International Journal of Enhanced Research in Science, Technology & Engineering. 2015;4(7):54-60.
[20]. Al-Ibrahim NSA, Hatim NA, Taqa AA, "Preparation of Local Gingival Shade Guide by Using Natural Pigments". IJERSTE. 2014;3(3):1-115.
[21]. Chu YH, Chang CL, Hsu HF, "Flavonoid content of several vegetables and their antioxidant activity". Journal of the Science of Food and Agriculture. 2000;80(5):561-566.
[22]. Wong SK, Lim YY, Chan EWC, "evaluation of antioxidant, anti-tyrosinase and antibacterial activities of selected hibiscus species". Ethnobotanical Leaflets. 2010;14: 781-796.
[23]. Oboh G, "The neuroprotective potentials of sour (hibiscus sabdariffa, calyx) and green (camellia sinensis) teas on some pro-oxidants induced oxidative stress in brain". Asain j. clin. Nutr. 2009:1(1):40-49.
[24]. Meraiyebu AB, Olaniyan OT, Eneze C, Anjorin YD, Dare JB, "Anti-inflammatory activity of methanolic extract of hibiscus sabdariffa on carrageenan induced inflammation in Wistar Rat". Ijpsi. 2013;2(3):22-24.
[25]. Saravanan D, lakshmi IA, Gobinath M, kumar BG, Priya S, Syamala E, Rahamathbee K, "Potential antioxidant, hypoglycemic and hypolipidemic effect of leaves of hibiscus platanifolius linn.". IJPSDR. 2011;3(3):236-240.
[26]. Khafaga AFA, "Molecular genetic identification of some egyptian hibiscus samples". J Am Sci. 2013;9(10):28-35.
[27]. Sayago-Ayerdi SG, Arranz S, Goai I, Serrano J, "Dietary fiber content and associated antioxidant compounds in roselle flower (hibiscus sabdariffa l.) beverage". J Agric Food Chem. 2007;55(19):7886-7890.
[28]. Olaleye, Tolulope M, "Cytotoxicity and antibacterial activity of Methanolic extract of Hibiscus sabdariffa". J. Med. Plants Res. 2007;1(1):9-13.
[29]. Shyamala BN, Naidu MM, Sulochanamma G, Srinivas P, "Studies on the antioxidant activities of natural vanilla extract and its constituent compounds through in vitro models". J Agric Food Chem. 2007;55(19):7738-7743.