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PHYTOCHEMICAL ANALYSIS AND COMPARATIVE ANTIOXIDANT STUDY OF HONEY, EXTRACTS OF SOME MEDICINAL PLANTS AND THEIR MIXTURE WITH HONEY

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ABSTRACT
Nowadays medicinal plants are used to cure various diseases due to their fewer side effects. Medicinal plants show anticancer, anti-inflammatory, antimicrobial, anti-tubercular, and antioxidant activities. In this study, we selected some medicinal plants such as *Citrus sinensis, Ficus racemosa, Vitis vinifera, Phoenix dactylifera, Carica papaya, and Honey* due to their high medicinal impact. In the present work, phytochemical analysis and comparative antioxidant study of honey, extracts of medicinal plants such as *Citrus sinensis, Ficus racemosa, Vitis vinifera, Phoenix dactylifera, Carica papaya* and their mixture with *Honey* in stoichiometric proportions were studied to find out their positive or negative impact on antioxidant activity after mixing.
Keywords: Phytochemical constituents, *Citrus sinensis, Ficus racemosa, Honey,* DPPH Assay, Antioxidant Activity.
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INTRODUCTION

Nowadays medicinal plants are used to cure various diseases due to their fewer side effects. Medicinal plants show anticancer, anti-inflammatory, antimicrobial, anti-tubercular, and antioxidant activities. In the present work, we selected some medicinal plants such as *Citrus sinensis, Ficus racemosa, Vitis vinifera, Phoenix dactylifera, Carica papaya, and Honey* due to their high medicinal impact. The general uses of these plants are mentioned here. *Citrus sinensis* is rich in vitamin C. Its fruit is used for its medicinal properties. It is used as a protective agent against heart diseases, cancer, anxiety, diabetes, typhoid, arthritis, malaria, and a number of diseases. It shows antioxidant, anti-inflammatory, anti-ulcer, and antimicrobial properties.[1] *Ficus racemosa* is used for a variety of medicinal purposes they show anti-inflammatory, antidiuretic, antioxidant, and anticancer properties. It is also useful in urinary tract infections.[2-3] *Vitis vinifera* are brighter grapes that may be used as healthy snacks. They are a good source of fiber which is used in the process of digestion. They reduce stomach issues. They help to relieve constipation. It also prevents anemia, maintains strong bones, protects our teeth, reduces the risk of cancer and heart diseases, and improves intestinal function. Regularly eating of it helps to maintain the body's health and prevent various diseases.[4-5] *Phoenix dactylifera* are highly nutritious rich in fibre and used as an antioxidant. They are helpful in healthy bowel movement, improving brain function, reducing the risk of cancer, and preventing microbial infection. It is also used as a natural sweetener.[6] *Carica papaya* is a well-known fruit in India that is rich in vitamin A and several other minerals. Different parts of the papaya plant are used for various medicinal purposes. The extract of papaya leaf is used in curing dengue fever. Its fruit is used as an anti-oxidant & anti-inflammatory and anti-cancer agent. It is also used in chronic diseases.[7-8] *Honey* is used as a natural sweetener. It is used as an anti-inflammatory, anti-bacterial, and antioxidant agent. It strengthens immune systems, nourishes our skin & face, and boosts our memory. It is also used to treat coughs, home remedy for dandruff, and for healing wounds.[9] In the present work, phytochemical analysis and comparative antioxidant study of honey, extracts of medicinal plants such as *Citrus sinensis, Ficus racemosa, Vitis vinifera, Phoenix dactylifera, Carica papaya* and their mixture with Honey in

stoichiometric proportions were studied to find out their positive or negative impact on antioxidant activity after mixing.

EXPERIMENTAL

Phytochemical Analysis

The phytochemical analysis was performed to find out the different phytoconstituents present in the extracts of *Citrus sinensis, Ficus racemosa, Vitis vinifera, Phoenix dactylifera, Carica* papaya, and Honey by using water as a solvent and their mixture with honey in stoichiometric proportion.

Table-1: Phytochemical Analysis of Extracts of *Citrus sinensis, Ficus racemosa, Vitis vinifera, Phoenix dactylifera, Carica Papaya, Honey* and their Mixture

S. No.	Phytoconstituents	*Citrus sinensis*	*Ficus racemosa*	*Vitis vinifera*	*Phoenix dactylifera*	*Carica, papaya*	*Honey*	Mixture of six test samples
1.	Carbohydrates	+	+	+	+	+	+	+
2.	Reducing sugar	+	+	+	+	+	+	+
3.	Steroid /Triterpenoid	+	-	+	+	-	-	+
4	Protein	-	-	-	-	-	-	-
5	Alkaloid	+	+	+	+	-	-	+
6.	Amino acid	-	-	-	-	+	+	-
7.	Flavonoids	+	+	+	+	+	+	+
8.	Tannins	+	+	+	+	+	+	+
9.	Coumarin Glycosides	+	+	+	-	-	+	+
10.	Anthraquinone Glycosides	-	+	+	-	+	+	+
11.	Cyanogenetic Glycosides	-	-	-	-	+	+	-
12.	Cardiac Glycosides	-	-	-	-	+	+	+
13.	Saponin	-	+	+		+	+	+
14.	Barberine	-	+	+	-	-	+	-
15.	Morphine	+	-	-	+	+	+	-
16.	Aconitine	-	-	-	-	-	-	-
17.	Brucine	+	+	+	+	+	+	+
18.	Connine	-	-	-	-	-	-	-
19.	Ergometrine	-	-	-	-	-	-	-
20.	Hesperidin	-	-	-	-	-	-	-
21.	Papaverine	+	-	-	+	-	+	+
22.	Phygostigmine	+	-	-	+	+	-	-
23.	Quinine	+	+	+	+	-	+	+
24.	Quinidine	-	-	-	-	-	-	-
25.	Soalsodine	-	-	-	-	-	-	-
26.	Cinchonine	-	-	-	-	-	-	-
27.	Cinchonidine	-	-	-	-	-	-	-

+ means present and – means absent

Materials and Methods

The fresh fruits of *Citrus sinensis, Vitis vinifera, Phoenix dactylifera, Carica papaya,* and *Honey* were purchased from the Local market of Akola, District Akola. *Ficus racemosa* fruits were collected from the tree located in the area of Godbole plots, Dabki Road Akola, District Akola. The fresh fruits of *Citrus sinensis, Ficus racemosa, Vitis vinifera, Phoenix dactylifera, and Carica, papaya* was taken (each of 25gms) and mixed with 100 ml of water by using a mixer grinder to make their solution and filter it. 25 ml of honey mixed with 100 ml of water to make their solution. These filtered solutions were used freshly for further study.

Sample Preparation

The following samples were prepared for the study of antioxidant properties:

S. No.	Name of Test samples	Proportion
1.	Fruits of *Citrus sinensis*	25 gm in 100 ml water
2.	Fruits of *Ficus racemosa*	25 gm in 100 ml water
3.	A mixture of fruits of *Citrus sinensis* and *Ficus racemosa*	1: 1 mixture
4.	Fruits of *Vitis vinifera*	25 gm in 100 ml water
5.	Fruits of *Phoenix dactylifera*	25 gm in 100 ml water
6.	A mixture of fruits of *Vitis vinifera* and *Phoenix dactylifera*	1: 1 mixture
7.	Fruit of *Carica papaya*	25 gm in 100 ml water
8.	*Honey*	25 ml in 100 ml of water
9.	A mixture of the fruit of *Carica papaya* and *Honey*	1: 1 mixture
10.	A mixture of fruits of *Citrus sinensis, Ficus racemosa, Vitis vinifera, Phoenix dactylifera, Carica papaya,* and *Honey.*	1:1 mixture of all

Antioxidant Activity by DPPH Assay Method

The antioxidant activity of aqueous extracts of i) fruits of Citrus sinensis and Ficus racemosa and their (1:1) mixture ii) fruits of Vitis vinifera and Phoenix dactylifera and their (1:1) mixture iii) fruit of Carica papaya and Honey and their (1:1) mixture iv) (1:1) mixture of aqueous extracts of fruits of Citrus sinensis, Ficus racemosa, Vitis vinifera, Phoenix dactylifera, Carica papaya, and Honey were analyzed by using the radical scavenging effect of the stable (1,1-diphenyl-2-picrylhydrazyl) DPPH assay method. The required solutions for the experiments were prepared by using water. DPPH used for this experiment is 0.04% in ethyl alcohol. 3 ml of DPPH solution was mixed with 3 ml of sample solutions. The test solutions were kept in the dark for half an hour and optical density was measured at wavelength 517 nm using an ultraviolet-visible spectrophotometer. DPPH solution (0.004%, 3 ml) and Ethanol (3 ml) were used as standard. The optical density was measured and Percent inhibition was calculated using the formula given below:[10-12]

$$\text{Percent Inhibition of DPPH (\% A. A.)} = \frac{\text{O. D. of blank} - \text{O. D. of Sample}}{\text{O. D. of blank}} \times 100$$

Where A.A. = Antioxidant activity and O. D. = Optical density.

RESULTS AND DISCUSSION

The test solutions required for the experiment were prepared as per the required dilutions and O. D. along with % A. A. activity were measured. Optical density for blank DPPH = 0.96.

Table-2: O. D. of Aqueous Extract of *Citrus sinensis, Ficus racemosa* and Their Mixture

Conc. mg/ml	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
O. D. of *Citrus sinensis*	0.92	0.89	0.88	0.86	0.83	0.82	0.80	0.75	0.72
O. D. of *F. racemosa*	0.75	0.72	0.62	0.60	0.58	0.57	0.53	0.50	0.45
O.D of *C. sinensis* and *F. racemosa* mixture	0.94	0.74	0.73	0.69	0.63	0.59	0.58	0.54	0.50

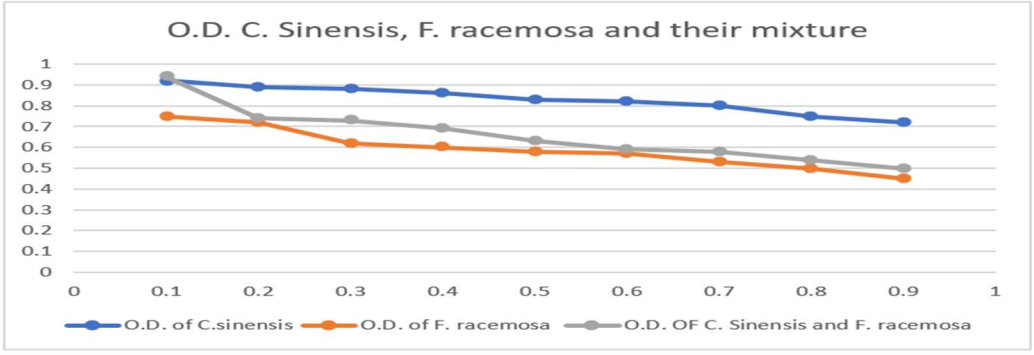

Fig.-1: Decrease in O. D. of the Test Sample with Increase in the Concentration of Aqueous Extract of *C. Sinensis, F. racemosa,* and Their Mixture

Table-3: % A. A. of Aqueous Extract of *Citrus sinensis, Ficus racemose*, and Their Mixture

Conc. mg/ml	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
% A. A. of *Citrus sinensis*	4.1	7.2	8.3	10.4	13.5	14.5	16.6	21.8	25.0
% A. A. of *F. racemosa*	21.8	25.0	35.4	37.5	39.5	40.6	44.7	47.9	53.1
% A. A. of *C. sinensis* and *F. racemosa* mixture	2.0	22.9	23.9	28.1	34.3	38.5	39.5	43.7	47.9

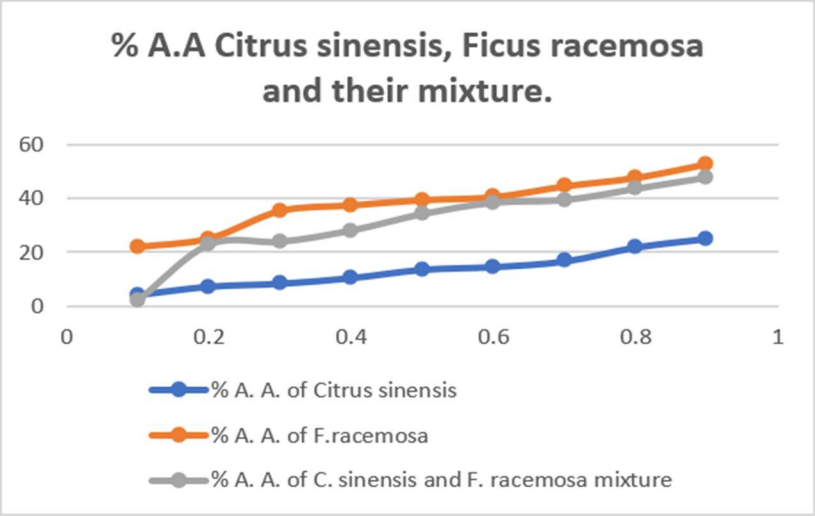

Fig.-2: Increase in % A. A. with Increase in Concentration of Aqueous Extract of *Citrus sinensis, Ficus racemose,* and Their Mixture

Table-4: O. D. for Aqueous Extract of *Honey, Carica Papaya,* and Their Mixture

Conc. mg/ml	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
O.D. of *Honey*	0.86	0.82	0.80	0.77	0.74	0.69	0.65	0.64	0.55
O.D. of *Carica papaya*	0.92	0.87	0.83	0.78	0.73	0.68	0.65	0.62	0.57
O.D. of *Honey* and *Carica papaya* mixture	0.81	0.79	0.74	0.72	0.70	0.68	0.67	0.63	0.61

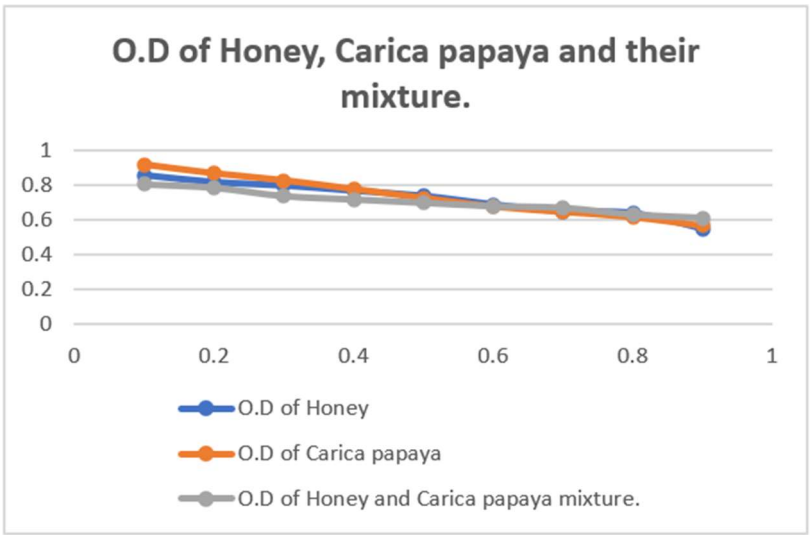

Fig.-3: Decrease in O. D. of Test Sample with Increase in Concentration of Aqueous Extract of *Honey, Carica Papaya*, and Their Mixture

Table-5: % A. A. for aqueous Extract of *Honey*, *Carica* Papaya, and Their Mixture

Conc. of mg/ml	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
% of A.A *Honey*	13.13	17.17	19.19	22.22	25.25	30.30	34.34	35.35	44.44
% of A.A *Carica papaya*	7.07	12.12	16.16	21.21	26.26	31.31	34.34	37.37	42.42

% of A.A Honey and Carica papaya mixture	18.18	20.20	25.25	27.27	29.29	31.31	32.32	36.36	38.38

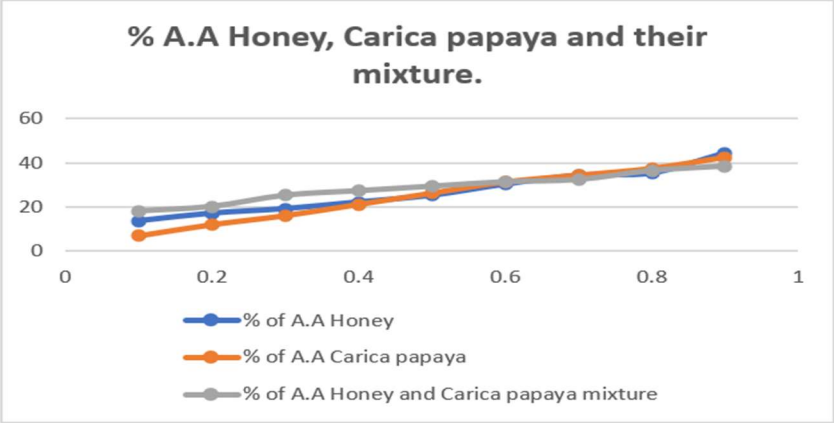

Fig.-4: Increase in % A. A. with an Increase in the Conc. of Aq. Extract of *Honey, Carica Papaya,* and their Mixture

Table-6: O. D. for Aqueous Extract of *Vitis vinifera, Phoenix dactylifera* and Their Mixture

Conc. mg/ml	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
O.D of V.vinifera	0.81	0.56	0.47	0.36	0.35	0.34	0.30	0.29	0.28
O.D of P.dactylifera	0.84	0.80	0.74	0.72	0.69	0.64	0.60	0.50	0.48
O.D of V. vinifera and P. dactylifera mixture	0.86	0.79	0.74	0.62	0.60	0.58	0.45	0.35	0.25

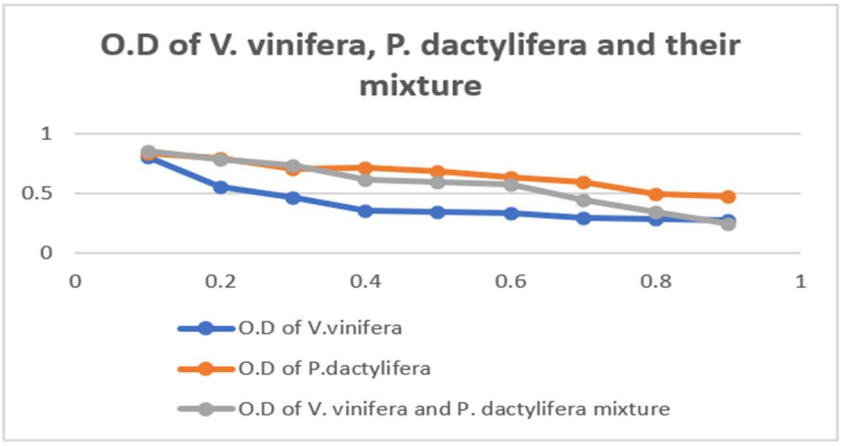

Fig.-5: Decrease in O. D. with Increase in Concentration of Aqueous Extract of *Vitis vinifera, phoenix dactylifera,* and Their Mixture

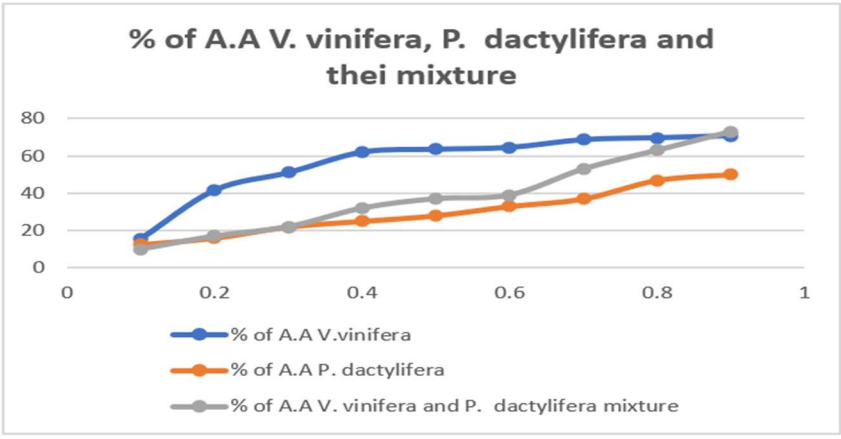

Fig.-6: Increase in % A. A. with an Increase in the Concentration of Water Extract of *Vitis vinifera, P.dactylifera* and Their Mixture

Table-7: % A. A. for Aqueous Extract of *Vitis vinifera, P.dactylifera* and Their Mixture

Conc. mg/ml	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
% of A.A V. vinifera	15.6	41.6	51.0	62.0	63.5	64.5	68.7	69.7	70.8
% of A.A P. dactylifera	12.5	16	22	25	28	33	37	47	50
% of A.A V. vinifera and P. dactylifera mixture	10	17	22	32	37	39	53	63	73

Table-8: Optical Density for Mixture of Aqueous *Extract of Citrus sinensis, Ficus racemosa, Vitis vinifera, Phoenix dactylifera, Carica papaya* and Honey

Conc.mg/ml	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
O.D of a mixture of six test samples	0.87	0.81	0.75	0.74	0.68	0.66	0.60	0.58	0.57

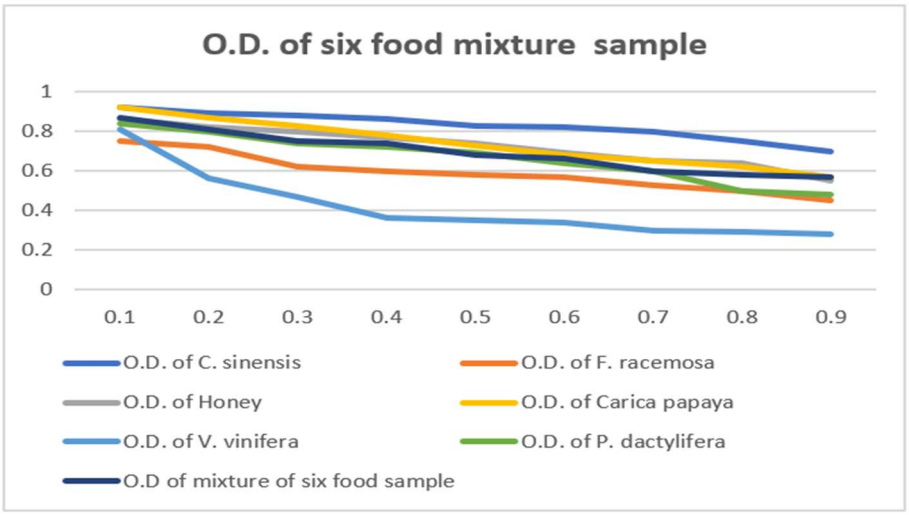

Fig.-7: Decrease in O. D. with Increase in Concentration for *extract of Citrus sinensis, Ficus racemosa, Vitis vinifera, Phoenix dactylifera, Carica papaya,* and Honey

Table-9: % A. A. for Extract of Mixture of Aqueous *Extract of Citrus sinensis, Ficus racemosa, Vitis vinifera, Phoenix dactylifera, Carica papaya* and *Honey*

Conc. mg/ml	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
% A.A of a mixture of six test samples	9.3	15.6	21.8	22.9	29.1	31.2	37.5	39.5	40.5

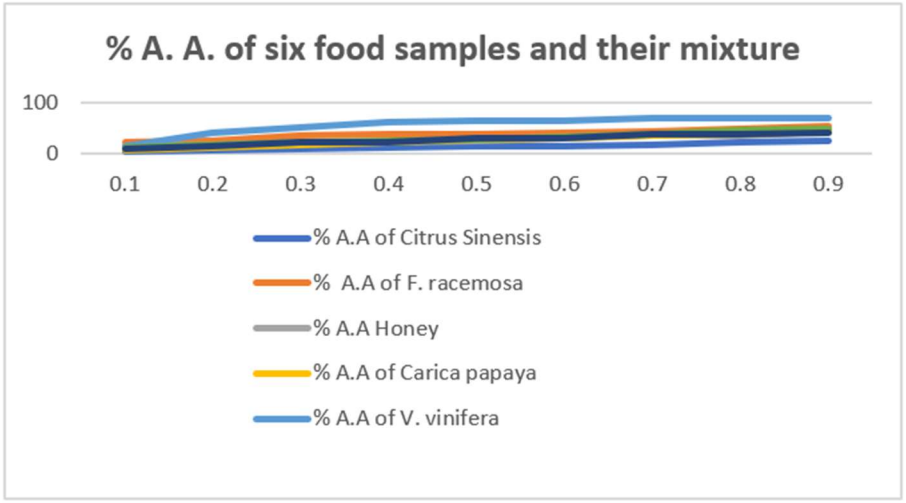

Fig.-8: Increase in % A. A. with Increase in Concentration for Extract of Mixture of Aqueous Extract *of Citrus sinensis, Ficus racemosa, Vitis vinifera, Phoenix dactylifera, Carica papaya,* and *Honey*

Table – 10: IC_{50} Values for Various Test Sample Extracts and Their Mixture

S. No.	Test Sample Extracts	IC50
1.	*Citrus Sinensis*	0.65
2.	*Ficus racemosa*	0.35
3.	Mixture of *Citrus sinensis* and *Ficus racemosa*	0.32
4.	*Honey*	0.45
5.	*Carica papaya*	0.43
6.	A mixture of *Honey and Carica papaya*	0.48
7.	*Vitis vinifera*	0.23
8.	*Phoenix dactylifera*	0.54
9.	A mixture of *Vitis vinifera* and *Phoenix dactylifera*	0.63
10.	Mixture of *Citrus sinensis, Ficus racemosa, Vitis vinifera, Phoenix dactylifera, Carica papaya* and *Honey*	0.32

CONCLUSION

The result for the analysis of phytoconstituents for the aqueous extract of i) fruits of *Citrus sinensis* and *Ficus racemosa* and their (1:1) mixture ii) fruits of *Vitis vinifera* and *Phoenix dactylifera* and their (1:1) mixture iii) fruit of *Carica papaya* and *Honey* and their (1:1) mixture *iv) (1:1) mixture of* aqueous extracts of fruits of *Citrus sinensis, Ficus racemosa, Vitis vinifera, Phoenix dactylifera, Carica papaya* and *Honey* were presented in Table-1. The results obtained for the study of antioxidant activity by DPPH assay method performed for test samples of i) fruits of *Citrus sinensis* and *Ficus racemosa* and their (1:1) mixture ii) fruits of *Vitis vinifera* and *Phoenix dactylifera* and their (1:1) mixture iii) fruit of *Carica papaya* and *Honey* and their (1:1) mixture *iv) (1:1) mixture of* aqueous extracts of fruits of *Citrus sinensis, Ficus racemosa, Vitis vinifera, Phoenix dactylifera, Carica papaya* and *Honey* were presented (Table-2 to 8). If we mix the various test samples having antioxidant activities then a certain mixture of test samples shows good antioxidant activities as compared to their individual samples. In the present study Mixture of *Citrus sinensis* and *Ficus racemosa* shows greater antioxidant activity as compared to their individual activity. Again, if we mix all the six test samples then we found a tremendous increase in antioxidant activity in the mixture as compared to their individual activities. A measurable decrease in Optical Density values of test sample extracts was observed from the graph, which clearly indicates good antioxidant activity. The IC_{50} values of the various test sample extracts were measured from the graphs which are given above.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/ editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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