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Green Synthesis of Gold Nanoparticles from *Manilkara zapota* L. Extract and Evaluation of its Intrinsic *In vivo* Anti-Arthritic Potential

Mahnoor Ijaz[1†], Maryam Fatima[2†], Rukhsana Anwar[2*], Maliha Uroos[1*]

[1] Centre for Research in Ionic Liquids, School of Chemistry, University of the Punjab, Quaid-e-Azam Campus, 54590 Lahore, Pakistan

[2] Punjab University College of Pharmacy, University of the Punjab Lahore, Pakistan

*Corresponding authors: malihauroos.chem@pu.edu.pk, rukhsana.pharmacy@pu.edu.pk

SUPPORTING INFORMATION

Table 1: *In vitro* anti-arthritic activity of aqueous leaf extract and nanoparticles of *Manilkara zapota.*

Groups	Conc (µg/ml)	Mean ± SD
Aqueous Extract	50	15.34 ± 1.2
	75	20 ± 1.23
	100	47.61 ± 2.19
	200	58.73 ± 3.61
	400	65.90 ± 2.47
Gold NPMZ	50	17.80 ± 1.15
	75	27.54 ± 2.15
	100	51.96 ± 2.15
	200	60.77 ± 3.84
	400	71.68 ± 1.95
Diclofenac Sodium	50	24 ± 0.54
	75	34 ± 1.26
	100	69.10 ± 0.66
	200	76.36 ± 1.71
	400	85.59 ± 2.19

Table 2: *Result of phytochemical analyses of Manilkara zapota leaf extract*

Serial No.	Chemical test for Phytochemical constituents	Results
1	Alkaloids	Positive
2	Terpenoids	Positive
3	Glycosides	Positive
4	Reducing sugars	Positive
5	Saponins	Positive
6	Carbonyl compounds	Negative
7	Tannins	Positive
8	Flavonoids	Positive
9	Steroids	Positive
10	Coumarins	Positive
11	Carotenoids	Positive
12	Sterols	Positive
13	Anthocyanins	Negative
14	Emodins	Positive
15	Phenols	Positive
16	Phlobatanin	Positive

Phytochemical Tests of *Manilkara zapota*

Various biochemical tests were performed in order to check the presence of various phytochemical constituents including alkaloids, saponins, coumarins, tannins, flavonoids and other poly-phenolic components.

1. Alkaloids

1g of aqueous extract was taken in the test tube. 2mL of 2% sulfuric acid was added into it and heated for two minutes. The solution was filtered. After that small number of drops of Dragendorff's reagent were poured into the test tube and checked the formation of orange red precipitates. Formation of orange red ppt. confirmed the presence of alkaloids in aqueous extract.

2. Terpenoids

2mL of chloroform along with 0.5g aqueous extract was added in the test tube. In order to form the layer conc. Sulfuric acid was added into it and observed the formation of reddish brown color. There was the formation of reddish brown color, which indicates the presence of terpenoids.

3. Glycosides

0.5g of aqueous extract was taken in the test tube. 2mL acetic acid was added in the test tube. After that a minute drop of ferric chloride solution was added and checked the formation of brown ring at the interface. A brown ring at the interface indicates the presence of glycosides.

4. Reducing sugars

3mL aqueous extract was taken in the test tube. Few drop of Fehling solution both A and B was added. The mixture was boiled for 2 minutes and observed the formation of orange red precipitates. Formation of orange red precipitates indicates the presence of reducing sugars.

5. Saponins

3mL of aqueous extract was taken in the test tube. The test tube was heated until it started to boil. The development of frothing indicated the presence of saponins

6. Carbonyl compounds

2mL aqueous extract was mixed with some drops of 2,4-dinitrophenylhydrazine. The mixture was shaken well and checked the immediate formation of yellow crystals to confirm the presence of aldehyde. The development of yellow color confirmed the presence of carbonyl compounds.

7. Tannins

3mL aqueous extract was heated in water bath and filtered. To the filtrate, neutral ferric chloride solution was added. The formation of dark green solution confirmed the presence of tannins.

8. Flavonoids

0.2g aqueous extract was weighed in a test tube. It was dissolved in 2ml of 10%NaOH and then diluted with HCl. To confirm the presence of flavonoids, observed the formation of yellow color in solution. The formation of yellow color confirmed the presence of flavonoids.

9. Steroids

0.5g aqueous extract was dissolved in 2mL of acetic acid and 2mL sulfuric acid was added into it. The color change from violet to green confirmed the presence of steroids.

10. Coumarins

3ml of 10%NaOH was dissolved in 2mL of aqueous extract and observed the formation of yellow color. The formation of yellow color indicated the presence of coumarins.

11. Carotenoids

The blue green color formation after the addition of few ml of phenol and HCl (1:1) to the aqueous extract was observed. It indicated the presence of carotenoids in aqueous extract.

12. Sterols

The aqueous extract was dissolved in chloroform and filtered. Drops of conc. Sulfuric acid was added to the filtrate and mixed. It was allowed to stand for few minutes and observed the development of red or yellow color in the lower layer which indicated its presence.

13. Anthocyanin

2mL of 10%NaOH was added to the small amount of aqueous extract and checked the development of any blue green color. The formation of blue green color confirmed the presence of anthocyanin.

14. Emodins

The formation of red color after the addition of 2mL NH_4OH and 3mL benzene to the aqueous extract was observed. It confirmed the presence of emodins.

15. Phenols

Solution of lead acetate was added into 1mL aqueous extract solution and observed the formation of precipitates, which confirmed the presence of phenols.

16. Phlobatanin

2mL aqueous extract was boiled with 1mL of 2% HCl solution and observed the formation of red precipitates.