بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

A study of Phytochemical and Antimicrobial Activity of *Citrus paradisi* and *Citrus* sinensis Seeds

دراسة فيتوكيمائية والفعالية البيولوجية لبذور نباتى القريب فروت والبرتقال

A thesis submitted in fulfillment of the requirement of the Degree of ``Master of Science`` in Chemistry

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Introduction

Plants have anchored to mother long before man set his feet on earth. God has endowed mankind with materials for survival much before his arrival on earth.

Availability of medicinal plants is not problem especially in developing countries. Orange and Grape fruit, the tasty, juicy fruits belonging to the family Rutaceae are botanically know as Citrus Sinensis (Orange) and Citrus Paradisi (Grape), they are most important and widely grown fruit crop, with total global production reported to around 120 million tons. The trees are widely cultivated in tropical and subtropical climate, for its tasty juice and medicinal value. In worldwide trades Citrus fruits generate about 105 billion dollars per year all over the world.

The fruits cultivated in more than 130 countries, including India, UK, France. They are generally available from winter through summer with seasonal variations depending on the variety.

Seeds are greenish to pale whitish Flattened and angular the seed is generally poly embryonic the embryos are either “zygotic”, or “nuclear” the zygotic embryos are derived from pollination of the ovary “i.e” sexual reproduction and therefore are not always similar in horticultural qualities to the parent tree. The nuclear embryos are derived wholly from the mother plant and show very similar characteristics to the parent plant.

Justifications:

Its being thought by many people that Orange and Grape are very usefull plants for health and beauty, no wonder that they are most popular fruits in the world.

Orange and Grape are well known in Sudan, the two fruits are cultivated in Nahr ElNil State. They are known for their nutritional and medicinal proprieties throughout the world, from times immemorial whole Orange and Grape plants including ripe and unripe fruits, juice, their peels, leaves and flower used in traditional medicine and beauty. They belong to the family Rutaceae. The major medicinal properties of them include anti bacterial, anti fungal.

Many pervious researches in Sudan concentrated on the phytochemical analysis of fruits, leaves, flowers, and other parts of these two fruits, but the seeds were not covered and they need to be analyzed for their chemical constituents.

Objectives:

1. Successive extraction of air cleaned seeds powder for orange and grape seeds.
2. Phytochemical analysis of crude extract for both two plant seeds.
3. Assessment of anti microbial of all extracts for two plant seeds
4. Determine the composition of both oil and crude extracts for grape and orange seeds

Material and Method

Collection of Plants materials:

Fresh plants will be collected from Khartoum or Nahr Elnil states, plants will be authenticating by Taxonomy Department.

Preparation of crude extract of plants:

Each of the coarsely powdered plant material (100) will be exhaustively extract for 20 hour with chloroform in Soxhlet apparatus. The chloroform extract will be filtered and evaporated under reduced pressure using Rota-Vap. The extract plant material will then air-dried, repacked in Soxhelt and exhaustively extracted with methanol. The methanolic extract will be filtered and evaporated under reduced pressure again using Rota-Vap. Each residue will be weighed and yield percentage will be determined. The chloroform residue (2) g will be suspended in a mixture containing methanol petroleum ether (2:1) to a final volume 20 ml (con. 100 mg/ml). the methanolic residue 2 g will be dissolved in methanol 20 ml (con. 100 mg/ml) and kept in refrigerator until used.

Phytochemical analysis of the extracted oil:

The phytochemical analysis of the extract will be done by UV, FTIR and / or HPLC.

In vitro testing of extracts for anti microbial activity:

After preparation of bacterial suspension, the antimicrobial activity of plant extracts will be tested using cup-plate agar diffusion method. The minimum inhibitory concentration will be determined by agar plate dilution method, and compared with commercial antibacterial.

Testing of Extracts for Antibacterial Activity:

The cup-plate agar diffusion method will be adopted according to (Khavanagh, 1972) to assess the antibacterial activity of prepared extracts.

0.6 ml of standardized bacterial stock suspension (10^8 – 10^9) colony forming units per ml will be thoroughly mixed with 60 ml of sterile nutrient agar. 20 ml of the

inoculated nutrient agar will be distributed into sterile petri dished. The agars will be left to set and in each of these plates 3 cups 10 mm in diameter, will be cut using cork N 04 and the agar discs will be removed. Alternate cups will be filled with 0.1 ml of each extracts using micro titer-pipette and will be allowed to diffuse at room temperature for two hours. Three replicates will be carried out for each extract against each of the test organism. Simulataneously addition of the respective solvent instead of extracts will be carried out as controls, after incubation the diameters of the results growth inhibition zones will be measured, averaged and the mean values will be tabulated.

Plan of work:

Activity	**Time / Month**																							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**
Sample collection																								
Extraction of sample																								
Phytochemical studies																								
Antimicrobail Activity																								
Literature review																								
Interpretational data and submission of thesis																								

Refereneses:

- Lukacin, R. A. L. , (2003) phytochemistry, 62, 287.
- Almagboal A. Z (1992), Antimicrobail and phytochemical investigation of vernonia amygdallina and other Sudanese Medicinal plants. Ph. D. Thesis. University of Khartoum, Sudan.
- Kavanagh, F. (1972) Analytical Mictobiology. Vol. 1. Academic press (pub). New York, And London.
- Parle Milind and Chaturvedi Dev (2012), Orange: Range of benefits, International Research Journal of Pharmacy ; 3(7) : 59 – 60.

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