Studies on Emblica Officinalis and Synthesis of Chalcones

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Abstract: Natural products obtained from the plants have a wide range of medicinal values. The pure chemical components separated from the plant extract provides unlimited opportunities for new drug as they have unmatched chemical diversity. Due to the increasing therapeutic use of natural products interest in edible plants has grown throughout the world. Chemical synthesis of the natural product is also carried out to learn a variety of biological activities. The focus of this paper is on methodologies that include extraction, separation, identification, and synthesis of natural products. The work was divided into two parts. Extraction of Fruits of Emblica Officinalis was carried out and three compounds were isolated and identified. In the second part synthesis of Chalcone was carried out. Two methods are reported for the synthesis of Chalcones in the literature i.e. Claisen-Schmidt condensation & Aldol condensation. However the synthesis of Chalcone has been done by using Aldol condensation of acetophenone and benzaldehyde. Different techniques such as chromatography i.e. Column chromatography and Thin Layer Chromatography (TLC) as well as spectroscopic techniques such as IR, UV, and NMR were used for identification of Natural & synthetic compounds.

***Keywords*: Extraction, Isolation, Synthesis & Natural products**

I. Introduction

A natural product is an organic substance which is produced from natural sources such as plants, animals or micro-organisms such as bacteria and fungi. Natural products play a vital role in health care for decades as they possess pharmacological activities that are useful in treating various kinds of diseases. These natural products may act as active components not only for traditional medicine but also for modern medicines. They are used as preliminary points for drug discovery in the chemical synthesis. Natural products played a prominent role in ancient traditional medicine system (Ayurveda, Chinese and Egyptian). The top drugs from the last century have been developed from natural products (Taxol, vincristine, and morphine).

Natural products include:

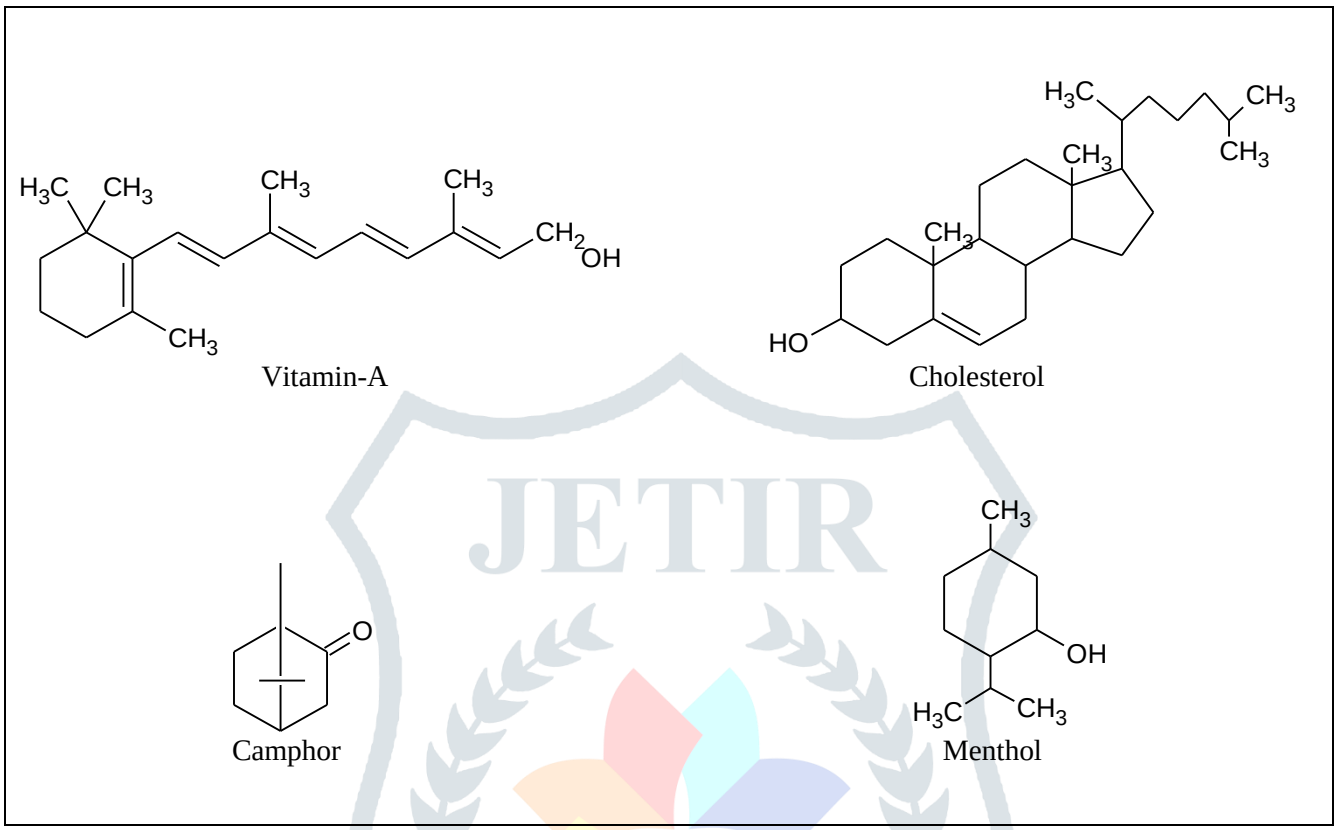
1. An organism that has not been exposed to any type of treatment other than drying.
2. An isolated animal organ flowers or leaves of plants.
3. Extract of an organism.
4. Pure compounds (alkaloids, glycosides, flavonoids, steroids, etc) isolated from plants, animals, and micro-organisms.

The natural products that are isolated can be further divided into primary and secondary metabolites. The primary metabolites include sugars, amino acids, nucleic acids, fatty acids whereas the secondary metabolites include more diverse compounds than primary metabolites. They refer to the compounds present in a specialized cell. Higher plants synthesize several compounds such as polyphenolic. Esters are present in the leaves of vascular plants whereas lignin and suberin are the examples of phenolics containing the polymer.

Phenol is a term that defines a phenyl ring bearing one or more hydroxyl substituent. The term polyphenolic is used to define the natural products that contain at least two phenyl ring bearing one or more hydroxyl substituents including the derivatives. The major class of phenolics in plants contains phenolics acids commonly represented as gallic acid, vanillic and syringic acid. Gallic acid is the basic unit of gallotannins whereas they are the subunits of ellagitannins. Tannins are a group of phenolics compounds

having a high molecular weight which can form the complex with carbohydrates and proteins. Plant polyphenolic plays a key role as a defense compound for environmental stresses such as low temperature, high light, pathogen infection, and nutrient deficiency.

Table 1.1 examples of natural products



All the Natural products occur as a mixture with other compounds from which the products of interest must be isolated.

Methods of isolation of natural products generally involve:

1. Extraction
2. Separation
3. Identification

1. Extraction

Extraction is a process in which the chemical components are extracted from the plant material which is then further separated and characterized. The basic steps include pre-washing, drying of plants materials, grinding to obtain a homogenous sample. The selection of the solvent depends on the nature of the compounds that are being targeted. The extraction of hydrophilic compounds is done by using polar solvents such as methanol, ethanol or ethyl acetate. For lipophilic compounds, a mixture of dichloromethane/methanol is used. In some cases, extraction is done using hexane. The extract is also prepared by maceration or dried powder plant material in organic solvent or water.

Modern techniques of extraction include pressurized liquid extraction, solid-phase micro-extraction, supercritical fluid extraction, surfactant mediated techniques. All these techniques have advantages such as reduced use of solvent consumption and solvent degradation, improvement in solvent efficiency, their selectivity and kinetics of extraction.

2. Separation

Plant extract occurs as a mixture of chemical compounds with their different polarities. Therefore separation is a big challenge for their identification and characterization. The methods used for the separation of mixture include Column chromatography, TLC after which the separated products are then used for the determination of their structure.

3. Identification

Identification of the separated compounds is done through spectroscopic techniques such as UV, IR, and NMR.

II. Materials and methods:

Melting points of the separated compounds were recorded in open glass capillaries and uncorrected. The chemical structures of the obtained compounds were confirmed by spectrometric methods such as UV and IR. Infra-red spectra in (KBr pallets) were recorded on Simadzu spectrophotometer. Thin Layer Chromatography was used to check the purity of the compounds

Part I: Isolation of Natural products

Procedure:

The prewashed fresh fruits (200 g) of Emblica Officinalis were extracted using ethyl acetate as a solvent. Extraction was carried out for 10 hours by reflux method. The extract was refluxed at 50℃.

Fig. 1 a*mla fruit*

Fig. 2 *reflux of amla*

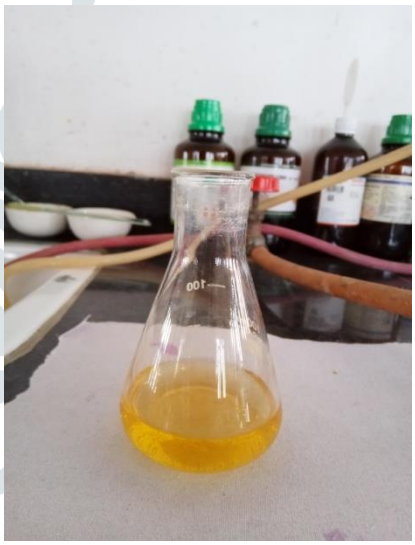


Fig. 3 *ethyl extract of amla*

Ethyl acetate extract

A small volume of ethyl acetate extract was concentrated which yielded a colorless crystalline solid at room temperature. It was crystallized from petroleum ether and its melting point was found to be 189-190 ℃. Its identity as vitamin-C was confirmed by its direct comparison with an authentic sample. The R_f value of the authentic sample and the obtained crystal were found to be the same.

The ethyl acetate extract was then concentrated to a small volume. The concentrated fraction was then column chromatographed over silica gel. The column was eluted with petroleum ether, petroleum ether with an increasing amount of benzene, pure benzene, benzene with an increasing amount of ethyl acetate successively. Subsequent elutions of solvents yielded fractions F_1 to F_5.

Fig. 4 e*lution of column*

Fig. 5 f*ractions collected through column*

Fraction F_1 contained Vitamin-C, F_4 contained Gallic acid, F_5 contained Ellagic acid whereas products from fraction F_2 and F_3 are obtained and their structures will be established further.

Fraction F_4

It was crystallized from methanol as a yellowish powder. Its melting point was found to be 234-240℃. It gave violet-black color with alcoholic ferric chloride.

Fig. 6 g*allic acid*

Fraction F_5

It was crystallized from aqueous pyridine which was filtered and washed with dilute HCl to remove pyridine and then with water. Its melting point was found to be 310 ℃. It gave bluish-green color with NaOH. It gave blood-red color on treating with nitric acid showing it to be ellagic acid. It was finally confirmed by comparing it with the authentic sample (superimposable IR).

Fig. 7 e*llagic acid*

Part II: Synthesis of Chalcone

Chalcone

Chalcones and their derivatives form a large group of natural products. Chalcones are the natural pigments found in nature which form the central core for a variety of important biological compounds. They are the aromatic ketones and enones. They are important constituents of many natural sources. They are found abundant in plants and are also considered as a precursor of flavonoids and isoflavonoids. They possess a wide spectrum of biological activities such as antibacterial, antifungal, anticancer, anti-inflammatory, etc. Some Chalcones have been reported as the inhibitors of lipoxygenase, β-secretase, acyl-cholinesterase, butyrylcholinesterase, cyclooxygenase, and peroxisome.

Chalcone shows biological activity mainly because of an enone pharmacophore in their structures. Cyclic ketones having α-hydrogen when treated with various aromatic aldehyde in alcohol in the presence of potassium hydroxide tends to form α, β-unsaturated compounds. Chalcones can be synthesized by using Claisen-Schmidt based catalyzed condensation of aromatic ketones or substituted aromatic ketones with benzaldehyde or substituted benzaldehyde.

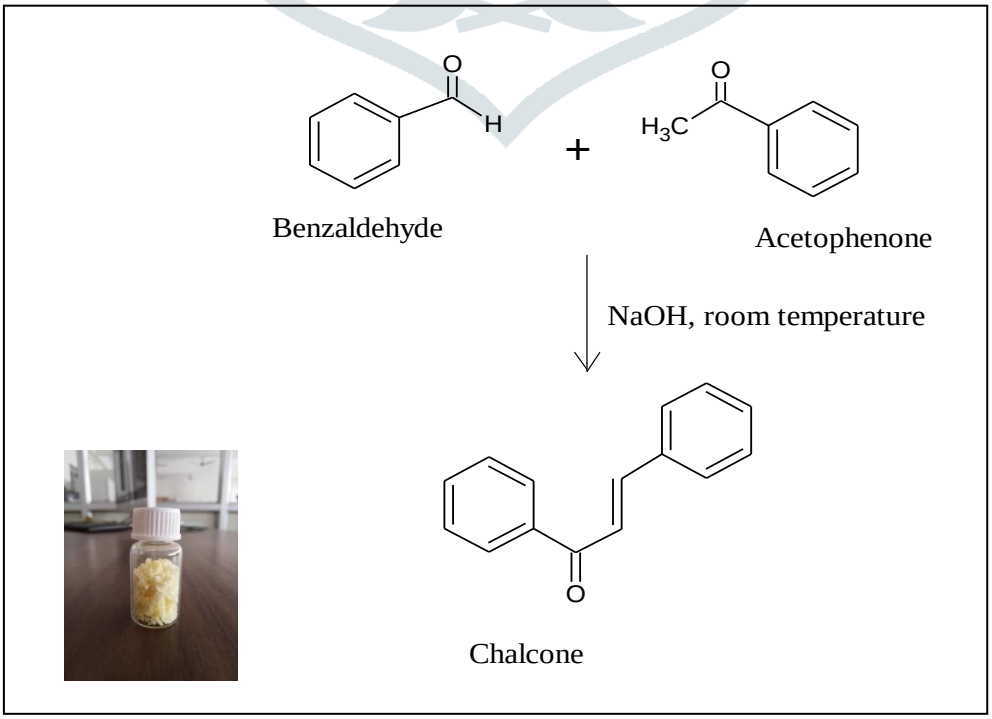
Chalcones are also known as Benzylidene acetophenone and it was first isolated from Chinese licorice (Glycyrrhiza inflate). It has a 1,3-diaryl-1-one skeletal system which was recognized as the main pharmacophore for chalcones. From plants, stable chalcone moiety cannot be isolated due to the presence of enzyme chalcone synthetase which immediately converts chalcone to flavanones.

Chalcone consists of two aromatic rings which are linked by an aliphatic three carbon chain. They are α, β-unsaturated ketones that consist of two aromatic rings having different substituents. These rings are interconnected by electrophonic three carbon α,β-unsaturated carbonyl system that assumes a linear or a planar structure and contain the keto-ethylenic group (-CO-CH=CH-).

Procedure:

Synthesis of Chalcones was carried out by using Aldol condensation. 20 ml Acetophenone and 20 ml Benzaldehyde were mixed in the presence of base NaOH as a catalyst. The reaction mixture was kept at 0-20^0 C temperature for 120 hours. The mixture was then filtered and washed with distilled water to remove access to raw materials. On completion of the reaction, it was monitored by TLC. Thin Layer Chromatography of reactants (Benzaldehyde and Acetophenone) and the obtained product Chalcone was carried out in hexane: ethyl acetate (12:2). It was sprayed with 5% H_2SO_4 where brown color Spot of the final product was observed. The reactant mixture was acidified with HCl in an ice bath and the solid was then filtered and crystallized by ethanol.

Scheme 1. Synthesis of Chalcone by Aldol Condensation



III. Results and Discussion: (Part I)

Gallic acid

Yellow powder crystallized from methanol. Melting point: 234-240 ̊C.

Spectral data:

λ_{max} – 220, 271 nm

KBr cm^{-1}: 3250 (OH), 1620 (C=O stretching), 1425, 1310, 1215, 1025, 900, 870, 770, 735 Cm^{-1}.

^{1}HNMR ($CDCl_3$, δ): 7.15 (2H, s, H-3 and H-7); s: singlet

^{13}C NMR ($CDCl_3$, δ): 167.4(C-1), 120.8(C-2), 109(C-3 and C-7), 145(C-4 and C-6), 137(C-5)

Ellagic acid

Brown powder crystallized from pyridine. Melting point: 310 ̊C.

Spectral data:

λ_{max} – 255nm

KBr cm^{-1}: 3550 (OH), 1740, 1720, 1620, 1520, 1430, 1360, 1220, 1100, 1060, 920 and 750 cm^{-1}.

^{1}HNMR ($CDCl_3$, δ): 7.45 (s, 2H, ArH), 10.67 (s, 4H, -OH)

Results and Discussion: (Part II)

Chalcone

Aldol condensation was carried out between acetophenone and benzaldehyde in the presence of sodium hydroxide to give a higher yield of Chalcone. The mixture was kept for 5 days at 0 ̊C. TLC shows two starting material and one final product. The final product was separated and crystallized for further test. The yield of the final product obtained was approximately 80%. The structure of the synthesized compound was confirmed by IR and NMR. It was observed to be yellow needles after crystallizing it through Ethanol. The melting point of 1, 3-Diphenyl propenone was found to be 56-57 ̊C.

Spectral data:

IR (KBr Cm^{-1}): 3068 (Ar=CH stretching), 1605.73 (C=C conjugation with C=O stretching), 1661 (CH=CH stretching), 1526 (Aromatic C=C vibration).

^{1}HNMR ($CDCl_3$, δ): 7.42-7.67 (8H, m), 7.80-7.45 (1H D, J=15.75Hz, 1.47Hz)

IV. Conclusion:

1. Five compounds were separated by Column chromatography and out of them three compounds were identified as Vitamin-C, Gallic acid and Ellagic acid. The identification of the structure of two compounds is in progress.
2. Chalcone was synthesized by using Aldol condensation and its structure was also established.

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References

1. K.H. Khan. “Roles of Emblica Officinalis in Medicine-A Review”, 2009
2. Newman DJ, Cragg GM, Sander KM. “ The Influence of Natural products upon Drug Discovery, Natural product Res 2000;17
3. Yokozawa, T., H.Y. Kim, H.J. Kim, T. Okubo, D.C. Chu, and L.R. Juneja, 2007. Amla prevents dyslipidemia and oxidative stress in the aging process. Br. J. Nutr., 97(6)

4. Alka N Choudhary, “Synthesis of Chalcone and their Derivatives as Antimicrobial agents”, (2011)
5. Prasad YR, Rao AL and Rambabu R. Synthesis and Antimicrobial Activity of some Chalcone Derivativ es. E-Journal of Chemistry, 2008; 5(3):461-466.
6. R. Jain, R. Pandey, R.N. Mahant and D.S. Rathore, “A Review on Medical Importance of Emblica Officinalis”.
7. Ashish Nagar, Ph. D thesis entitled "Synthetic and Natural studies of some plant polyphenolic", (1978)153-154