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Preliminary Studies on Biotransformation of Drumstick (*Moringa oleifera*) and Watermelon (*Citrullus lanatus*) Seed Oils using Baker's Yeast

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Abstract: In this study, preliminary investigations on biotransformation of under utilized *Moringa oleifera* and *Citrullus lanatus* seed oils were carried out using baker's yeast (*Saccharomyces cerevisiae*). Biotransformation reactions were performed for 48 h using baker's yeast with *M. oleifera* or *C. lanatus* seed oil (experimental) and without yeast (control) in nutrient broth medium. After 48 h, products were extracted with hexane. The transformation of *Moringa oleifera* and *Citrullus lanatus* seed oils was identified by High Performance Thin Layer Chromatography (HPTLC). The HPTLC peaks demonstrated that the baker's yeast transformed the *M. oleifera* and *C. lanatus* seed oils into other metabolites. These results reveal that the drumstick and watermelon seed oils can be used to transform into other metabolites, which may be useful as starting materials for the synthesis of other specialty chemicals.

Key words: Biotransformation, *M. oleifera*, *C. lanatus*, seed oil, *Saccharomyces cerevisiae*, HPTLC

INTRODUCTION

Biotransformation is a process involving the use of microorganisms as catalysts to perform transformations of chemical compounds. Currently, biotransformation reactions are the subject of increasing interest in the pharmaceutical industry because of the demand for enantiomerically pure compounds (Schulze and Wubbolts, 1999) and it is also useful technique for producing medicinal and agricultural important chemicals from both active and inactive products.

Biotransformation methods that involve microbial or enzymatic biocatalysts, when compared to their chemical counterparts, offer the advantages of high selectivity and mild operating conditions. Use of biocatalysts also minimizes the problems of isomerization, racemization, epimerization and rearrangement that are common in chemical processes (Patel, 2000). Microbial transformation is one of the most attractive approaches for introducing functional groups into various inaccessible positions of organic compounds (Smith, 1984). Microbial transformation is one of the simplest and most direct methods for the preparation of a range of optically active compounds of moderate to high enantiomeric purity. Biochemical transformation of organic compounds to generate new compounds by specific type of fungus and bacteria is reported in literature (Roberts *et al*., 1995).

Moringa oleifera is commonly known as Drumstick, belonging to the single genus family Moringaceae, is a small fast-growing ornamental tree originally belongs to India. Root,

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bark, pods and leaves of this tree has been reported to possess anti-cancer, hypotensive, anti-arthritic and anti-inflammatory activities. Pods and young leaves of the plant are primarily used for vegetative purpose. *Moringa oleifera* seed contains 40% by weight of oil and the oil contains high percent of oleic acid. Seed oil is used for their antibiotic and anti-inflammatory properties to treat arthritis, rheumatism, gout, cramp, sexually transmitted diseases and boils. Detailed information on the nutritional value and chemical composition of *M. oleifera* seed oil is available in the literature (Lalas and Tsakins, 2002).

Citrullus lanatus commonly known as Watermelon is cultivated throughout arid regions of India. The fruits are supposed to quench the thirst of human beings in this arid zone. Watermelon seeds are oil-bearing but the oil content varies from 15–45%. The seeds are chiefly used as a masticatory, medicine, food and oil.

In recent years there is considerable interest in utilizing abundantly available natural resources such as vegetable and fruit oils as renewable feedstocks in the preparation of useful chemicals. Plant oils are rich sources of many types of naturally occurring compounds, including mixtures of glycerides, fatty acids, glycerol, tocopherols and various sterols, as potential chemical feedstocks (Ayhan, 2007).

Baker's yeast has gained considerable attention as a catalyst for biotransformation processes, especially involving carbon-carbon bond formation and oxidation reduction reactions, because yeast is so inexpensive and easy to obtain (Servi, 1990). Biotransformation of vegetable oils, steroids and other organic compounds using *Saccharomyces cerevisiae* has been reported in the literature (El-Sharkawy *et al*., 1992; Suresh *et al*., 2009; Elzbieta and Tomasz, 2007).

The aim of present investigation was to transform the under utilized *Moringa oleifera* and *Citrullus lanatus* seed oils into other metabolites using baker's yeast.

MATERIALS AND METHODS

Media and Microorganism

In this study nutrient broth medium was used. The nutrient broth medium, comprised of 15 g of sucrose, 0.2 g of $NaNO_3$, 0.05 g of K_2HPO_4, $MgSO_4.7H_2O$, KCl and 0.001 g of $FeSO_4.7H_2O$ prepared in distilled water (1 L).

Drumstick and watermelon seed oils were purchased from a local market (Chennai, India). The commercial Baker's yeast *Saccharomyces cerevisiae* (Safe Yeast Co. Pvt. Ltd., Mumbai, India) cells were used during this study. Present investigation was carried out in the year 2008. All the chemicals used in this study were of analytical grade.

Biotransformation of Drumstick and Watermelon Seed Oils

Two sets of 500 mL Erlenmeyer flasks were taken. To one set of flasks containing 90 mL of nutrient broth medium, 10 mL of drumstick or watermelon seed oil was added which served as control (without baker's yeast). To the second set of flasks containing 90 mL of nutrient broth medium supplemented with 10 mL of drumstick or watermelon seed oil (dissolved in 2 mL of acetone), 2 g of baker's yeast was added (experimental flasks). Each experiment was conducted in replicates. Both the sets of flasks were incubated at room temperature (27±1°C) on a shaker (200 rpm) and the fermentation was allowed to proceed for 48 h. Then both the flasks (experimental and control) were extracted separately with hexane and air dried.

High Performance Thin Layer Chromatography (HPTLC) Analysis of Products

High Performance Thin Layer Chromatography (HPTLC) was used to identify the transformation products from the hexane extracted samples. Thin Layer Chromatography

(TLC) was performed for the hexane extracted samples (experimental and control) using pre coated silica gel aluminum plates (7×4 cm size) (Merck, Germany). The samples were spotted using capillary tubes (1 mm) and elution was carried out using the chromatographic tank (10×6 cm) filled with developing solvent system of diethyl ether: hexane (3: 7). After elution, TLC plates (experimental and control) were removed and allowed to air dry. Then TLC plates were subjected to scanning (450 nm) using CAMAG HPTLC spectrophotometer to identify the transformation of seed oils (CAMAG HPTLC spectrophotometer with a scanner II densitometry and a Linomat IV applicator).

RESULTS

Results of biotransformation at the end of 48 h in the control flasks (containing seed oil without baker's yeast in the growth medium) showed no change in seed oil while the experimental flask containing drumstick or watermelon seed oil with baker's yeast in the growth medium showed the transformed product. The HPTLC analysis of extracted experimental sample of *M. oleifera* seed oil produced seven major peaks (Table 1), having Rf values of 0.04, 0.07, 0.11, 0.29, 0.62, 0.67 and 0.70 with purity of 9.72, 10.36, 39.11, 10.58, 13.92, 7.75 and 8.56%, respectively. Whereas control sample of *M. oleifera* seed oil produced six peaks (Table 1), which are significantly different to that of experimental sample peaks. Figure 1 shows the HPTLC peaks obtained for control and experimental samples of drumstick seed oil. The peaks obtained for experimental sample of drumstick seed oil were not corresponding with the control sample peaks (Fig. 1). This is due to biotransformation activity of baker's yeast in the experimental sample. Transformation was not occurred in control sample because of the absence of baker's yeast.

Table 1: Rf values and percent area of peaks of *M. oleifera* seed oil control and experimental samples

No. of peak	Rf max.	Area (%)
Control		
1	0.01	8.11
2	0.04	8.49
3	0.07	10.50
4	0.12	26.01
5	0.31	20.46
6	0.70	26.43
Experimental samples		
1	0.04	9.72
2	0.07	10.36
3	0.11	39.11
4	0.29	10.58
5	0.62	13.92
6	0.67	7.75
7	0.70	8.56

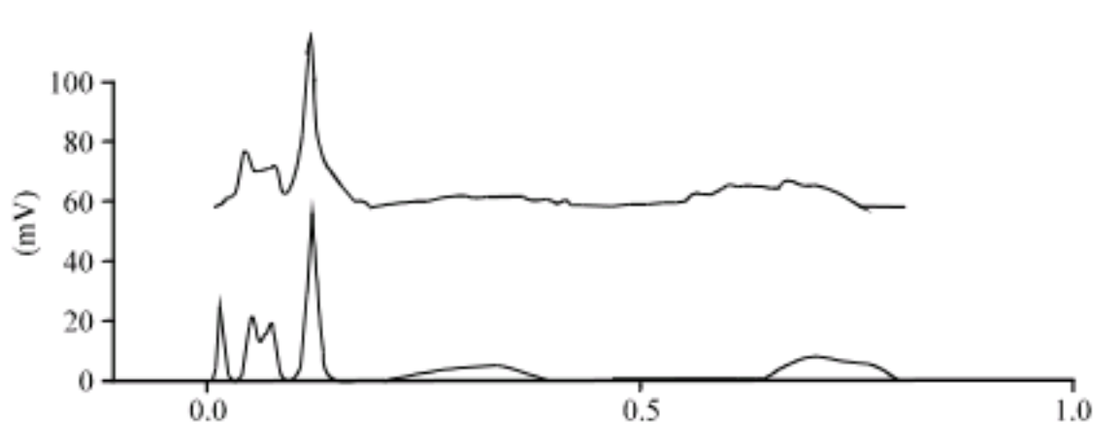

Fig. 1: HPTLC Chromatogram showing the peaks of Moringa seed oil (a) control and (b) experimental samples

Table 2: Rf values and percent area of peaks of *C. lanatus* seed oil control and experimental samples

No. of peak	Rf max.	Area (%)
Control		
1	0.03	2.79
2	0.10	8.93
3	0.28	25.97
4	0.60	37.87
5	0.59	24.44
Experimental samples		
1	0.04	0.69
2	0.09	12.34
3	0.30	41.87
4	0.59	45.10

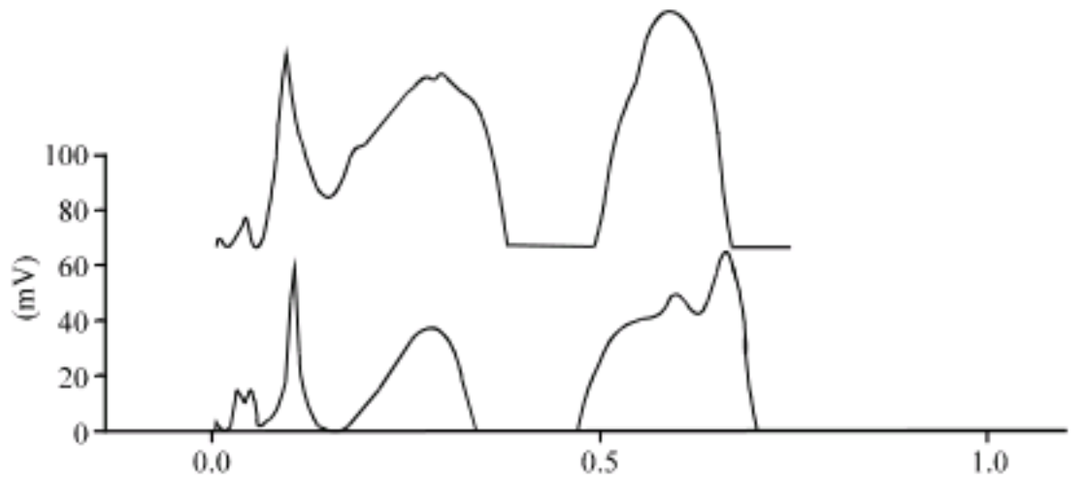

Fig. 2: HPTLC Chromatogram showing the peaks of watermelon seed oil (a) control and (b) experimental samples

Table 2 presents the Rf values and percent area of peaks obtained for control and experimental samples of *C. lanatus* seed oil. On examining the *C. lanatus* seed oil experimental (with baker's yeast) and control samples (without baker's yeast) using HPTLC, four major peaks were obtained for experimental sample (Table 2), having Rf values of 0.04, 0.09, 0.30 and 0.59 with purity of 0.69, 12.34, 41.87 and 45.10%, respectively. But these peaks were absent in control sample (Fig. 2). It confirms that the transformation of *C. lanatus* seed oil into other metabolites in experimental sample. These experiments proved that transformation of *M. oleifera* or *C. lanatus* seed oil has occurred due to enzymatic activity of baker's yeast.

DISCUSSION

Earlier reports are available in the literature on biochemical activity of microorganisms to transform organic compounds into other metabolites (Holland *et al*., 1995; Vollbrecht *et al*., 1998; Borges *et al*., 2007).

Long and Ward (1989) and Long *et al*. (1989) reported the ability of *Saccharomyces cerevisiae* to transform organic compounds into various products. Nikolova and Ward (1991) have also been reported the production of L-phenylacetyl carbinol by biotransformation using *Saccharomyces cerevisiae*. Young and Ward (1991) studied the reductive biotransformation of selected carbonyl compounds by whole cells and extracts of baker's yeast. El-Sharkawy (1996) reported the biotransformation of sunflower vegetable oil into useful chemicals by *Rhizopus stolonifer*. However, the biotransformation of *M. oleifera* or *C. lanatus* seed oil using *Saccharomyces cerevisiae* has never been reported. The results obtained in the present study further supported the biochemical activity of baker's yeast in transforming the organic compounds.

Biotransformation of *Moringa oleifera* and *Citrullus lanatus* seed oils in experimental flasks (containing baker's yeast) and no transformation in control flasks (without baker's yeast) was direct evidence to show that the baker's yeast cells aided in the transformation of *M. oleifera* and *C. lanatus* seed oils.

CONCLUSIONS

From the data presented in this study, it was found that baker's yeast (*Saccharomyces cerevisiae*) cells possess biochemical ability to perform transformation of *M. oleifera* and *C. lanatus* seed oils in to other metabolites. Based on these observations, further research on elucidation of mechanism of biotransformation and structural information of metabolites is recommended for the better utilization of these oils.

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