

# Phytochemical, Antioxidant Activity and Determination of Genetic Diversity in *Artocarpus Heterophyllus* Using RAPD Molecular Markers

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**Abstract:** The present investigation was aimed at analysing the phytochemical and antioxidant activity of leaves of 5 different Jackfruit sp. And Molecular characterization using 2 arbitrary primers with the help of RAPD technique, in order to understand the diversity among the various Jackfruit sp. The antioxidant property was evaluated using the radical scavenging assay. In the present study 4 different solvents were used for extraction of phytochemicals from leaf samples. Important phytochemicals such as alkaloids, carbohydrates and cardiac glycosides were determined in the leaf extracts using all the solvents. Acetone extract of the Jackfruit leaves exhibited good antimicrobial activity against the selected human pathogens i.e. *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The selected primers generate distinctive products. A total of 57 fragments were produced from the two RAPD primers used. The maximum number of fragment bands were produced by the OP A8 primer (GTGACGTAGG) was (32) bands which showed 21.87% polymorphism and 6.25% monomorphism and by the OP A9 primer (GGGTAACGCC) was (25) bands which showed 24% polymorphism and 3.84% monomorphism which indicates high diversity among the selected leaf samples. Polymorphism detected by randomly amplified polymorphic DNA (RAPD) has proved to be useful for identifying variation at different levels in the plants.

**Keywords:** Jackfruit, phytochemical, antioxidant activity, antimicrobial activity, Molecular characterization

## 1. Introduction

Plants which have been selected for medicinal use over thousands of years constitute the most obvious choice of examining the current search for therapeutically effective new drugs such as anticancer drugs (Dewick, 1996), antimicrobial drugs (Phillipson and Wright, 1996) and antihepatotoxic compounds. Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids (Edoga et al., 2005; Mann, 1978). These compounds are synthesized by primary or rather secondary metabolism of living organisms. A large number of phytochemicals belonging to several chemical classes have been shown to have inhibitory effects on all types of microorganisms in vitro (Cowan, 1999).

The jackfruit (*Artocarpus heterophyllus* Lam.) belonging to family Moraceae is an integral part of common Indian diet and commonly known as "Kathal". The yellowish bulbs constituting the perianth portion of the fruit are fleshy, fibrous, and rich in sugars as well as carotenoids. It is considered a rich source of carbohydrates, minerals, carboxylic acids, dietary fiber, and vitamins such as ascorbic acid and thiamine (Rahman et al., 1999). Different classes of flavonoids are abundant in the jack fruit plant (Lin et al., 2000; Wei et al., 2005). A major protein, Jacalin has been isolated from jackfruit seeds and possessed immunological properties (Kumar et al., 1982; Pereira-da-Silva et al., 2006). Soong and Barlow have evaluated antioxidant properties of jackfruit seeds and found to show more than 70% contribution to the total antioxidant activity and phenolic content.

Jackfruit includes hundreds of varieties, thousands of cultivars diversification based on the jacalin (lectins) concentration in the fruits. Among DNA based molecular markers, Random Amplified Polymorphic DNAs (RAPDs) provide an excellent tool for studying genetic relationships. Polymorphism detected by randomly amplified polymorphic DNA (RAPD) has proved to be useful for identifying variation at different levels in various plants (Hardys et al., 1992; Stiles et al., 1993; Khadari et al., 1995). In the present work was performed to investigate various phytochemicals present, antioxidant activity, antimicrobial activity of leaves of 5 different Jackfruit sp. Molecular characterization was also performed using 2 arbitrary primers in order to understand the diversity among the various Jackfruit sp

## 2. Materials and Method

### 2.1 Sample Collection

In the present study, both young leaves and old leaves of Jackfruit sp. were collected in zip lock bags separately from University of Agriculture Sciences, GKVK Campus, The Department of Plant Biotechnology, Bangalore, India. Five different Jackfruit sp. namely NSP-4, NSP-5, NSP-6, and NSP-35 & NSP-36 were collected.

The young leaves were stored in sterile bags at - 20 °C, which were further used for DNA isolation. The older leaves were used for phytochemical, antioxidant and antimicrobial assays.

## 2.2 Phytochemical Analysis

### 2.2.1 Preparation of Plant Extract

The older leaves of the selected Jackfruit sp. washed under running tap water to remove dust. The plant samples were then air dried for few days and the leaves were crushed into powder and stored in polythene bags for use. The powdered samples were subjected to extraction with solvents such as petroleum ether, acetone, hexane and water (in 1:10 ratio). 2 g of the powdered plant sample was weighed and poured with 20 ml of the respective solvent and incubated overnight for extraction. The solution was then filtered with the help of filter paper and filtered extract of the selected plant samples were taken and used for further phytochemical analysis like Alkaloids, Carbohydrates, Glycosides, Terpenoids, Proteins, Saponins, Phenols, Tannins, Flavonoids and Quinones.

### 2.2.2 Assays

(2, 2'-Azinobis -3-ethyl benzothiazoline-6-sulfonic acid) ABTS radical scavenging assay:

The antioxidant capacity was estimated in terms of the ABTS<sup>+</sup> radical scavenging activity following the procedure described by Delgado-Andrade et al. Briefly ABTS<sup>+</sup> was obtained by reacting 7mM ABTS stock solution with 2.45mM potassium persulfate and the mixture was left to stand in the dark at room temperature for 12-16 h before use. The ABTS<sup>+</sup> solution (stable for 2 days) was diluted with 5mM phosphate buffered saline (pH 7.4) to an absorbance at 730nm of 0.70 ± 0.02. After the addition of 10µl of sample to 4ml of diluted ABTS<sup>+</sup> solution, the absorbance was measured at 30 min. All samples were analyzed in triplicate. The ABTS<sup>+</sup> radical – scavenging activity of the samples was expressed as S% =  $\frac{A_{Control} - A_{Sample}}{A_{Control}} * 100$ , where A<sub>Control</sub> is the absorbance of the blank control (ABTS<sup>+</sup> solution without test sample) and A<sub>Sample</sub> is the absorbance of the test sample.

## 3. Molecular Characterization Using RAPD Technique

### 3.1 DNA Isolation

Genomic DNA was extracted from the young leaves of the collected *Jackfruit sp.* using the CTAB method following the procedure of Doyle and Doyle (1987). The leaves (0.05 g) were ground in a mortar with a pestle. Extraction buffer (1% (w/v) CTAB, 50 mM Tris-HCl (pH 8), 0.7 M NaCl, 0.1% β-mercaptoethanol) was added and the solution was incubated at 60 °C for 30 minutes. The homogenate was mixed with 25:24:1 phenol: chloroform: isoamyl alcohol

(v/v/v) by gentle inversion. After centrifugation at 13,000 rpm for 15 minutes, the upper aqueous layer was transferred to a fresh tube. The extraction of DNA with phenol/chloroform/isoamyl alcohol was repeated one more time. DNA in the solution was precipitated with double the volume of ice-cold isopropanol and washed twice with 70% ethanol. The obtained DNA pellet was dissolved in 50 – 100 µL sterile deionized water and stored at 4°C.

### 3.2 Estimation of DNA

#### 3.2.1 Qualitative estimation of DNA

The quality of the DNA was estimated using Agarose gel electrophoresis. The obtained DNA was run on 0.8 % Agarose gel stained with Ethidium bromide at 80 – 90 V for 1 hour followed by viewing the gel under UV trans illuminator.

#### 3.2.2 Quantitative Estimation of DNA

The quantity of the isolated DNA was further estimated by measuring the OD 260 and OD 280 or absorbance ratio. DNA yield and quality was calculated using a NanoDrop ND-1000 spectrophotometer (ThermoScientific) at 260 nm.

### 3.3 PCR Amplification using RAPD Markers

The five DNA samples were subjected to PCR amplification with 2 RAPD primers namely OP A8 and OP A9. The total volume of the PCR reaction was 25 µL, which contained 1 µL of template DNA, 2.5 µL of 10x Taq buffer with MgCl<sub>2</sub> (10 mM), 1.5 µL of 2.5 mM dNTPs, 2 µL of Taq polymerase (Chromous), and 1 µL of 10 pmol RAPD primer. The PCR cycle was carried out with the initial denaturation at 94 °C for 2 min followed by 36 cycles of 94 °C for 45 s, 37 °C for 1 minute, 72 °C for 1 minute and a final extension of 72 °C for 10 minutes. The obtained PCR product was viewed in 1.5% agarose gel stained with ethidium bromide.

## 4. Results

### 4.1 Phytochemical Analysis

From the results of phytochemical analysis it was observed that important medicinal phytochemicals such as Alkaloids, Cardiac glycosides and Carbohydrates were present in all sample extracts. The other phytochemicals found in various solvents are tabulated below (Table 1)

**Table 1:** Phytochemical Analysis

S.No.	Sample	Sap	Fla	Phe	Qui	Ter	Tan	Alk	Carb	C.Gly	Ptns.
1.	Water NSP 4	+ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve
	Water NSP 5	+ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve
	Water NSP 6	+ve	+ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve
	Water NSP35	+ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve
	Water NSP36	+ve	+ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve
2.	Petroleum Ether NSP 4	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve	-ve
	Petroleum Ether NSP 5	-ve	-ve	-ve	-ve	+ve	-ve	+ve	+ve	+ve	-ve
	Petroleum Ether NSP6	-ve	-ve	-ve	-ve	+ve	-ve	+ve	+ve	+ve	-ve
	Petroleum Ether NSP35	-ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve	+ve	-ve
	Petroleum Ether NSP36	-ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve	+ve	-ve

3.	Acetone	NSP 4	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve
	Acetone	NSP 5	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve
	Acetone	NSP 6	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve
	Acetone	NSP35	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve
	Acetone	NSP36	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve	+ve
4.	Hexane	NSP 4	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	+ve	+ve
	Hexane	NSP 5	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve
	Hexane	NSP 6	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve
	Hexane	NSP35	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	+ve	+ve
	Hexane	NSP36	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	+ve	+ve

Sap –Saponins, Fla-Flavonoids, Phe-Phenol, Qui-Quinones, Ter-Terpenoids, Tan-Tannin, Alk-Alkaloid, Carb-Carbohydrate, C.Gly-Cardiac Glycoside and Ptn-Protein.

#### 4.2 Antioxidant Assay

The percentage of scavenging activity was studied by using ABTS assays for *Artocarpus heterophyllus* family leaf of aqueous extract of NSP35 has 95 to 98%, NSP4 has 80 to 90%, NSP36 has 35to 94%, NSP6 has 6to 91% and NSP5 has 3to92%.For acetone extract percentage of scavenging activity of NSP4 was 40 to 80%,NSP5 19 to 66% ,NSP6 13 to 48 % ,NSP35 6 to 50 % and NSP36 12 to 58%.As a standard ascorbic acid has been used for ABTS assay. Out of all these extracts maximum % of inhibition was measured by water extract of NSP35 and in acetone extract NSP4 with 40 to 80 %.But in case of petroleum ether extract ABTS reagent was immiscible there was one layer formation just after adding ABTS reagent. At present date no one has done antioxidant assay on petroleum ether extract by using ABTS assay for *Artocarpus heterophyllus* leaf which is the extraordinary thing in this present paper. Maximum antioxidant assay has been done on *Artocarpus heterophyllus* leaf extract of methanol, water, ethanol, ethyl acetate using ABTS, DPPH, FRAP and etc. assays but not on petroleum ether extract (Figure-1a, 1b).

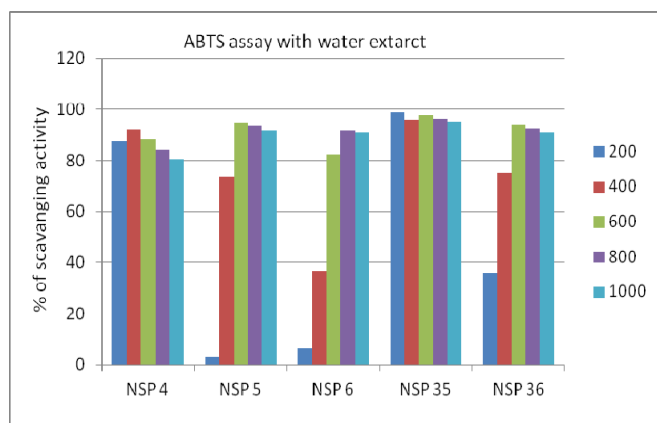


Figure-1(a): ABTS Assay with Water Extract

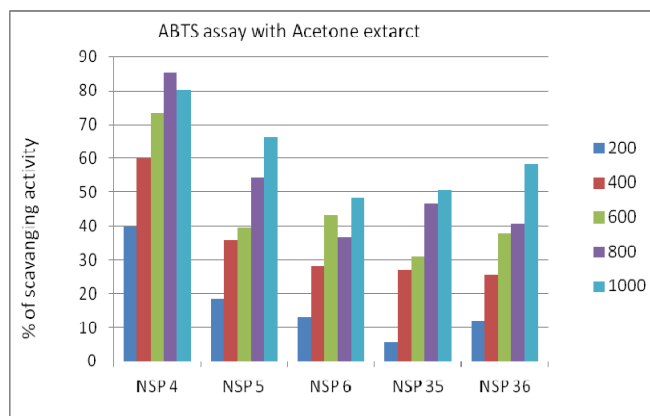


Figure 1(b): ABTS Assay with Acetone extract

#### 5. Molecular Characterization using RAPD Technique

The qualitative analysis of DNA was estimated using Agarose gel electrophoresis as shown in Figure 2. The obtained DNA showed sharp single bands on 0.8% agarose gel without any degradation or RNA contamination.

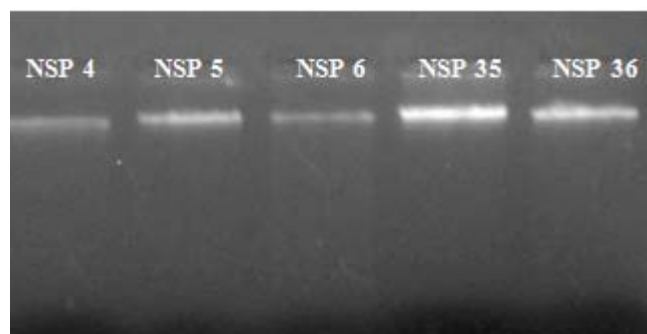


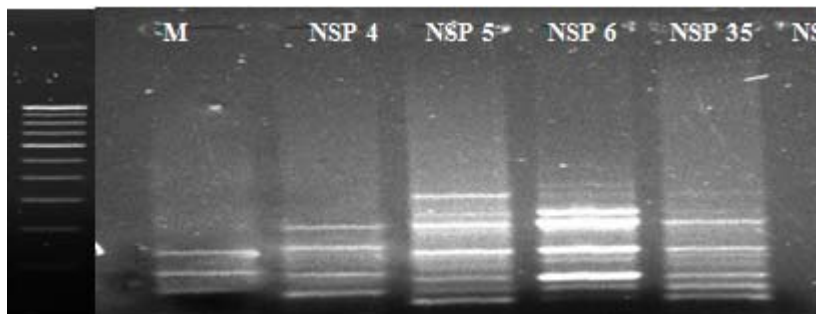
Figure 2: Isolated DNA of the various *Jackfruit sp.*

#### 4.3 PCR Amplification using RAPD Markers

In the present study, for RAPD- PCR two primers namely OP A8 and OP A9 were used. The amplification observed using both the primers are shown in Figure 3 and 5. The banding pattern score was based on the presence or absence of clear, visible and reproducible bands. The results were analyzed based on the principle that a band is considered to be ‘polymorphic’ if it is present in some individuals and absent in others, and ‘monomorphic’ if present in all the individuals. In this study, both the primers produced a total of 32 and 25 bands respectively Table-3.

**Table 3:** Polymorphism and monomorphism

Primer	Total no. of bands	% Polymorphism	% Monomorphism	Unique Bands
OP A8	32	21.87	6.25	2
OP A9	25	24	3.84	1



**Figure 3:** Amplification using OP A8

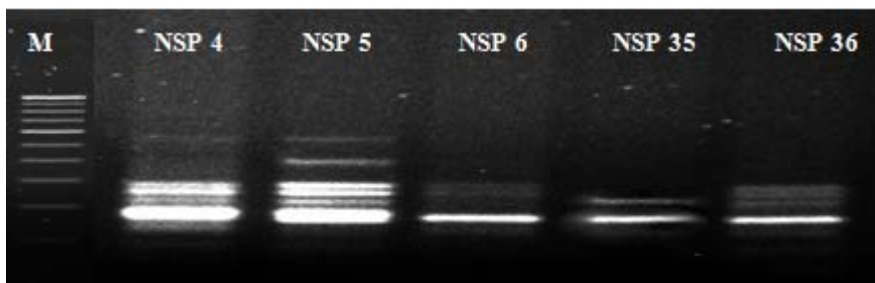
Among the bands obtained using primer OP A8, 21.87 % were polymorphic, 6.25 % were monomorphic and 2 unique bands were observed. The obtained RAPD data was further utilized for construction of dendrogram and similarity matrix Figure-4.



**Figure 4:** Dendrogram constructed by using OP A8 primer. 1 - NSP4, 2 -NSP5, 3 - NSP6, 4 - NSP35, 5 - NSP36

	1	2	3	4	5
1	100.00	81.82	72.73	9.09	72.73
2	81.82	100.00	72.73	9.09	72.73
3	72.73	72.73	100.00	36.36	100.00
4	9.09	9.09	36.36	100.00	36.36
5	72.73	72.73	100.00	36.36	100.00

The dendrogram obtained using primer OP A8 it was observed that, NSP 4 showed 81.82% similarity with NSP 5, whereas least similarity was observed between NSP 35 and NSP 4 (9 %).



**Figure 5:** Amplification using OP A9

Among the bands obtained using primer OP A9, 24 % were polymorphic, 3.84 % were monomorphic and 1 unique band was observed. The obtained RAPD data was further utilized for construction of dendrogram and similarity matrix Figure-6.



Figure 6: Dendrogram constructed by using OP A9 primer. 1 - NSP4, 2 -NSP5, 3 - NSP6, 4 - NSP35, 5 - NSP36

	1	2	3	4	5
1	100.00	6.25	18.75	12.50	0.00
2	6.25	100.00	87.50	93.75	93.75
3	18.75	87.50	100.00	81.25	81.25
4	12.50	93.75	81.25	100.00	87.50
5	0.00	93.75	81.25	87.50	100.00

The dendrogram obtained using primer OP A9, maximum similarity was observed between NSP 5 along with NSP 35 and NSP 36 (93.75 %), whereas least similarity (0%) was observed between NSP 4 and NSP 36.

## 6. Discussion

### 6.1 Phytochemical Analysis

The phytochemical analysis of these five different plant leaf species of same family namely *Artocarpus heterophyllus* was performed using standard protocol for qualitative determinations. The study indicated that terpenoid was present only in petroleum ether extracts of all plant species except NSP4 and protein was found only in hexane extract of all plants as well as in acetone extract of NSP36. Saponins and flavonoids are present only in aqueous extract of all plants. In all other aqueous extract of these plants tannins, alkaloids, phenol and cardiac glycosides were present except in NSP6. It should be noted that tannins and alkaloids were important compounds which gives antibacterial activity. All the plant extracts except petroleum ether and hexane doesn't contain tannin. Out of all the five plant leaf extract studied, only these compounds tannins, alkaloids, carbohydrate and cardiac glycosides were commonly extracted by using all water, petroleum ether, and hexane and acetone solvents. Rest of the compounds were extracted by different –different solvents. Till now very few phytochemical analyses has been done on *Artocarpus heterophyllus* leaf extract. Few of them have done phytochemical analysis on *Artocarpus heterophyllus* leaf of methanol and aqueous extract.

Ajayi I. A., Ajibade O. and Oderinde R. A., 2011 studied the phytochemical constituents from the seeds of *Artocarpus communis*, *Artocarpus heterophyllus*, *Calophyllum inophyllum*, *Garcinia kola*, *Garcinia man gostana*, *Pentaclethra macrophylla* and *Treulia Africana* and reported that all the plant specimens were found to contain flavonoids and reducing compounds but none of them contained carotenoid and steroids. Tannins were present in

all the samples except *Artocarpus communis*, saponins was not found only in *Artocarpus heterophyllus* and terpenoids was not found only in *Artocarpus communis* which is similar to our findings.

### 6.2 Antioxidant Assays

Deepika Gupta, K et al., 2011, investigated the phytochemical and nutritional property of seeds of jack fruit and proved that they were a good source of nutritional and antioxidant components and hold their potential for value addition and nutraceutical development.

Haidy S et al., 2011 examined the antioxidative, hypoglycemic, and hypolipidemic activities of *Artocarpus heterophyllus* leaf extracts. The ethanol and butanol extract showed scavenging activity for diphenylpicrylhydrazyl radical and chelate Fe<sup>2+</sup> in vitro.

Meshram R. L et al., 2011 19. evaluated in vitro antioxidant activity of *Balanites aegyptiaca* And *Artocarpus heterophyllus* using DPPH method. Acetone extract from the seed of *Artocarpus* showed an antioxidant activity of 12.34 %.

### 6.3 RAPD

RAPD were screened with the five different plant leaf species of same family *Artocarpus heterophyllus*. The pattern of RAPD fragments produced by the 10 - mer primers OP A8 and OP A9 are shown. The two informative primers were selected and used to evaluate the degree of polymorphism and genetic relationships within all the different species of *Artocarpus heterophyllus* under study. The selected primers generate distinctive products. A total of 57 fragments were produced from the two RAPD primers used. The maximum number of fragment bands were produced by the OP A8 primer (GTGACGTAGG) was (32) bands which showed 21.87% polymorphism and 6.25% monomorphism and by the OP A9 primer (GGGTAACGCC) was (25) bands which showed 24%

polymorphism and 3.84% monomorphism.

The dendrogram based on similarity matrix were constructed by using the Unweighted Pair Group of Arithmetic Means (UPGMA). The Dendrogram constructed by similarity matrix by using OP A8 primer shows two major clusters. The first cluster comprises of NSP36, NSP6, NSP4 and NSP5. The second cluster consists of one species of the NSP35. The sample 5 (NSP36) is closely related to sample3 (NSP6) and sample 1 (NSP4) is related to sample 2 (NSP5). The sample 4(NSP35) is highly distinct. On the other hand the dendrogram obtained from OP A9 primer shows two major cluster. The sample 2, sample 4 & sample 5 shows least variation within the different species of *Artocarpus heterophyllus* and sample 1 is distinct.).

## References

- [1] Clinical and Laboratory Standards Institute, "Performance standards for antimicrobial susceptibility testing," Nineteenth informational supplement M100-S19, Clinical and Laboratory Standards Institute, Wayne, Pa, USA, 2009.
- [2] Cowan, M.M. 1999. Plant products as antimicrobial agents. *Clin. Microbiol. Rev.* 564-582.
- [3] Deepika Gupta, Sonia Mann, Avijit Sood And Rajinder K. Gupta\*(2011), *phytochemical, nutritional and antioxidant activity evaluation of seeds of jackfruit (artocarpous heterolphyllus lam., University School of Biotechnology, GGS Indraprastha University, Dwarka, New Delhi 110075, India, International Journal of Pharma and Bio Sciences.p.337-339.*
- [4] Dewick, P.M. 1996. Tumor inhibition from plants: Tease and Evans.
- [5] Edoga, H.O., Okwu, D.E., Mbaebie, B.O. 2005. Phytochemicals constituents of some Nigerian medicinal plants. *Afr. J. Biotechnol.*, 4(7): 685-688.
- [6] Hadrys H., Balick M. & Schierwater B. (1992). Applications of random amplified polymorphic DNA (RAPD) in molecular ecology. *Molecular Ecology* 1:55-63.
- [7] Haidy S. Omar, Hesham A. El-Beshbishy, Ziad Moussa, Kamilia F. Taha, and Abdel Nasser B. Singab (2011), *Antioxidant Activity of Artocarpus heterophyllus Lam. (Jack Fruit) Leaf Extracts: Remarkable Attenuations of Hyperglycemia and Hyperlipidemia in Streptozotocin-Diabetic Rats*, *The Scientific World Journal* ,p. 788–800.
- [8] Khadari B., Lashermes P. & Kjellberg F. (1995). RAPD fingerprints for identification and genetic characterization of fig (*Ficus carica* L.) genotypes. *Journal of Genetics & Breeding* 49: 77-86
- [9] Kumar GS, Appuktan PS and Basu DK,  $\alpha$ -D-Galactose - specific lectin from jack fruit seed. *J Biosci*, 4: 257-261, (1982).
- [10] Lin, C.N., Lu, C.M., and Huang, P.L. (2000) Flavonoids from *Artocarpus heterophyllus*. *Phytochemistry* 39(6), 1447–1451.
- [11] Mann, J.1978. Secondary Metabolism. Oxford University press, London, pp. 154.
- [12] Meshram R. L., Umbarkar S.N.(2011), Comparative evaluation for in vitro Antioxidant activity from *Artocarpus heterophyllus* Lamk and *Balanites aegyptiaca* L., Department of Biochemistry, Shri Shivaji college of Arts, Commerce & Science, Akola, MS-India, *International Journal of PharmTech Research*, p.2006-2010.
- [13] Pereira-da-Silva G, Moreno A, Marques F, Oliver C, Jamur MC, Panunto-Castelo A and Roque-Barreira MC, Neutrophil activation induced by the lectin KM+ involves binding to CXCR2. *Biochim Biophys Acta*, 1760(1): 86-94, (2006)
- [14] Phillipson, J.D., Wright, C.W. 1996. *Plants With Antiprotozoal Activity : Tease and Evans, Pharmacognosy*, 14th edn., WB Saunders Company, London, pp. 612.
- [15] Rahman, M., Nahar, N., Jabbar, M., and Mosihuzzaman, M. (1999) Variation of carbohydrate composition of two forms of fruit from jack tree (*Artocarpus heterophyllus* L.) with maturity and climatic conditions. *Food Chem.* 65, 91–97.
- [16] Soong YY and Barlow PJ, Antioxidant activity and phenolic content of selected fruit seeds. *Food Chem*, 88: 411-417, (2004).
- [17] Stiles J.I., Lemme C, Sondur S., Morshidi M.B. & Manshardt R. (1993). Using randomly amplified polymorphic DNA for evaluating genetic relationships among papaya cultivars. *Theoretical & Applied Genetics* 85: 697-701.
- [18] Wei, B.L., Weng, J.R., Chiu, P.H., Hung, C.F., Wang, J.P., and Lin, C.N. (2005) Anti-inflammatory flavonoids from *Artocarpus heterophyllus* and *Artocarpus communis*. *J. Agric. Food Chem.* 53(10), 3867–3871.