

PROGRESS REPORT

EXTRACTION & PRELIMINARY PARTITIONING

Sorghum bicolor dried and ground leaves as supplied by Health Forever Products was extracted with 50% aqueous ethanol for 24hrs, with thorough agitation. The extract was subsequently filtered through cotton wool and concentrated *in vacuo* to give the crude extract designated as **(J)**.

The crude extract **(J)** was dissolved in 80% aqueous ethanol and partitioned into ethyl acetate and *n*-butanol successively. The ethyl acetate and *n*-butanol fractions were concentrated *in vacuo* to give fractions **(JE)** and **(JB)** respectively. A portion of the left over aqueous fraction was obtained was freeze-dried to give **(JA)** fraction.

FRACTIONATION & ISOLATION OF COMPOUNDS

From the results of the previous COX-inhibition bioassay done by Health Forever and experience, the ethyl acetate fraction (JE) was sub-fractionated on a silica gel (230-400 mesh) column using medium pressure liquid chromatographic technique using a gradient of *n*-hexane (200 ml), a doubling gradient of 100ml each of EtOAc-*n*-hexane, EtOAc, doubling gradient of MeOH-EtOAc and finally MeOH. Fractions of 15 ml each were collected in test tubes and monitored by thin layer chromatography (TLC) with normal phase silica gel using Ether/hexane (96:4) and 20% EtOAc in ether as the solvent systems. Fractions, which showed the same TLC characteristics, were bulked as appropriate and concentrated *in vacuo* to give 8 major fractions JE-1 to JE-8 (Fig. 1).

Repeated column chromatography of JE-4 on silica *n*-hexane (100 ml), a doubling gradient of EtOAc-*n*-hexane, EtOAc, doubling gradient of MeOH-EtOAc, MeOH), Sephadex LH-20 column, and open column on silica with a gradient of ether in hexane afforded compounds JE-43, JE-44, JE-45 and JE-47 as pure compounds. Similar treatment of JE-5 yielded 2 semi pure fractions coded JE-51 and JE-52. The pure compounds are being subjected to spectroscopic analysis.

COX-INHIBITION TESTING

The following fractions J, JE, JB, JA, JE-5, JE-6, JE-7 and JE-8 were subjected to COX-1 and COX-2 inhibition bioassays at Brunswick laboratories, England. The results and the inferences deducible from the results are as in Table I attached. Fraction JE-4 has also been submitted for testing.

FIG 1: FLOW CHART FOR ISOLATION OF COX INHIBITORS FROM JOBELYN RAW MATERIAL

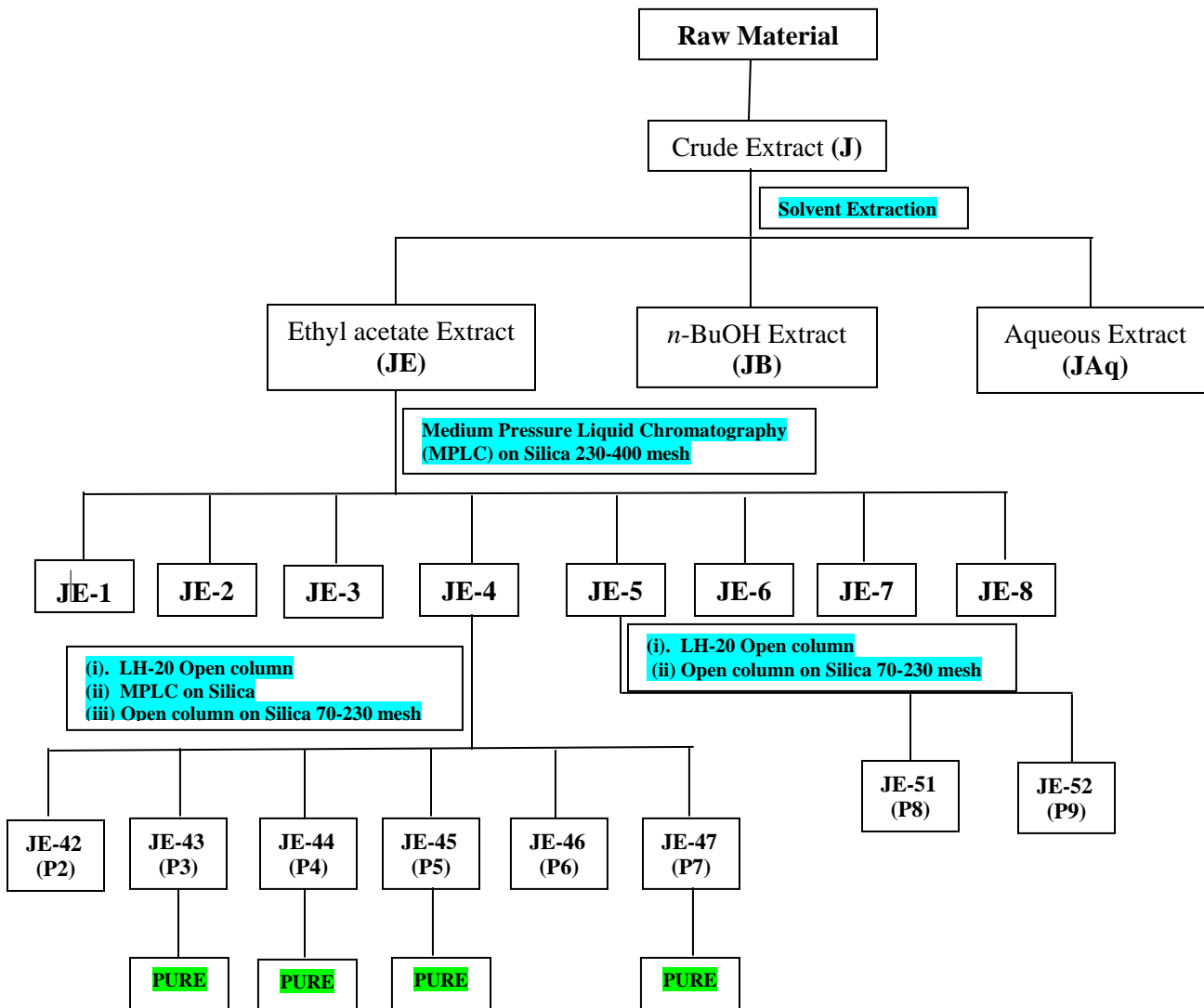


TABLE 1: RESULTS FROM TESTED FRACTIONS FOR COX-1 AND COX-2 INHIBITION.

S/No	Sample ID	Brunswick Lab ID	COX-1 IC ₅₀ (mg/ml)	COX-2 IC ₅₀ (mg/ml)	COX-1 IC ₅₀ /COX-2 IC ₅₀	COX-2 IC ₅₀ /COX-1 IC ₅₀
1.	J	07-2732	0.0053	0.0865	0.0613	16.3207
2.	JA	07-2733	0.0028	0.1154	0.0243	41.2143
3.	JB	07-2734	0.0033	0.0076	0.4342	2.3030
4.	JE	07-2735	0.0063	0.0102	0.6176	1.6191
5.	JE-5	07-2736	0.0238	0.0118	2.0169	0.4958
6.	JE-6	07-2737	0.0067	0.0074	0.9054	1.1044
7.	JE-7	07-2738	0.0073	0.0254	0.2874	3.4794
8.	JE-8	07-2739	0.0057	0.0450	0.1267	7.8947

INFERENCES.

1. The crude fraction (J) and all the major sub-fractions (JE, JB and JA) have good COX-1 activity at IC₅₀ values between 2.8 and 6.3µg/ml. Similarly, all the column fractions of JE that were tested equally have good COX-1 activity except fraction JE-5.
2. It is also good to note that the COX-2/COX-1 ratio for JE-5 is below 1 (highlighted in blue). This shows that this fraction contains compounds that possibly have COX-2 selective inhibition activity. The next logical step is to concentrate on purifying compounds from JE-5 and JE-6, both of which look promising for COX-2 selective inhibitor.

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